Therapeutic TB vaccine

Cross-Reference to Related Applications

This application is a non-provisional of US Patent Application No. 60/401,725, filed August 7, 2002, and claims the benefit of the priority thereof.

Background of the Invention

The present invention discloses a therapeutic vaccine against latent or active tuberculosis infection caused by the tuberculosis complex microorganisms (*Mycobacterium tuberculosis.*, *M.bovis, M.africanum*). The invention furthermore discloses a multi-phase vaccine that can be administered either prophylactically or therapeutically as well as a diagnostic reagent for the detection of latent stages of tuberculosis.

Human tuberculosis caused by *Mycobacterium tuberculosis* (*M. tuberculosis*) is a severe global health problem, responsible for approx. 3 million deaths annually, according to the WHO. The worldwide incidence of new tuberculosis (TB) cases had been falling during the 1960s and 1970s but during recent decades this trend has markedly changed in part due to the advent of AIDS and the appearance of multidrug resistant strains of *M. tuberculosis*.

Organisms of the tuberculosis complex can cause a variety of diseases, but the commonest route of invasion is by inhalation of bacteria. This initiates an infection in the lung, which can ultimately spread to other parts of the body. Normally, this infection is restricted in growth by the immune system, so that the majority of infected individuals show few signs apart from cough and fever, which eventually abates. Approximately 30% of individuals are unable to contain the infection and they will develop primary disease, which in many cases will eventually prove fatal. However, it is believed that even those individuals who apparently control the infection remain infected, probably for the rest of their life. Certainly, individuals who have been healthy for years or even decades can suddenly develop tuberculosis, which has proven to be caused by the same organism they were infected with many years previously. *M. tuberculosis* and other organisms of the TB complex are unique in that the mycobacteria can evade the immune response and survive for long periods in a refractory

non-replicating or slowly-replicating stage. This is referred to as latent TB and is at present a very significant global health problem that is estimated to affect approximately 1/3 of the world's population (Anon., 2001).

The course of a *M. tuberculosis* infection runs essentially through 3 phases, as illustrated in figure 1. During the acute phase, the bacteria proliferate in the organs, until the immune response increases to the point at which it can control the infection, whereupon the bacterial load peaks and starts declining. After this, a latent phase is established where the bacterial load is kept stable at a low level. In this phase *M. tuberculosis* goes from active multiplication to dormancy, essentially becoming non-replicating and remaining inside the granuloma. In some cases, the infection goes to the reactivation phase, where the dormant bacteria start replicating again. The full nature of the immune response that controls latent infection and the factors that lead to reactivation are largely unknown. However, there is some evidence for a shift in the dominant cell types responsible. While CD4 T cells are essential and sufficient for control of infection during the acute phase, studies suggest that CD8 T cell responses are more important in the latent phase. It is also likely that changes in the antigen-specificity of the response occur, as the bacterium modulates gene expression during its transition from active replication to dormancy.

The only vaccine presently available for clinical use is BCG, a vaccine whose efficacy remains a matter of controversy. Although BCG consistently performs well in animal models of primary infection, it has clearly failed to control the TB epidemic. Consistent with that, BCG vaccination appears to provide protection against pediatric TB (which is due to primary infection), while offering little or no protection against adult disease (which is often reactivation of latent infection acquired in childhood). It has also been shown that vaccination of individuals who are currently sensitized to mycobacteria or latently infected is ineffective. Thus, current vaccination strategies, while effective against primary disease, fail to activate immune responses that efficiently control surviving dormant bacteria.

At this point no vaccine has been developed that confers protection against reactivation whether given as a prophylactic vaccine prior to infection or as a therapeutic vaccine given to already latently infected individuals.

This makes the development of a new and improved vaccine against TB an urgent matter, which has been given a very high priority by the WHO. Many attempts to define protective mycobacterial substances have been made, and different investigators have reported increased resistance after experimental vaccination. However, these efforts have almost exclusively focused on the development of prophylactic vaccines for the prevention of disease (Doherty, 2002), and such vaccines have not been demonstrated to work if given in an immunotherapeutic fashion (Turner, 2000).

It has been suggested that the transition of *M. tuberculosis* from primary infection to latency is accompanied by changes in gene expression (see, for example, Honer zu Bentrup, 2001, which is incorporated herein by reference). *In vitro* hypoxic culture conditions, which mimic the conditions of low oxygen tension and restricted nutrients found in the granuloma (the location of the latent infection), have been used to analyze changes in gene expression and a number of antigens have been found that are induced or markedly upregulated under these conditions e.g. the 16 kDa antigen α-crystalline (Boon, 2001, Monahan, 2001, Florczyk 2001, Sherman 2001, Manganelli, 2001, all of which are incorporated herein by reference) and Rv0569 as described in Rosenkrands, 2002, and which is described in WO0179274.

As noted in the references cited above, it is already known that some genes are upregulated under conditions that mimic latency. However, these are a limited subset of the total gene expression during latent infection. Moreover, as one skilled in the art will readily appreciate, expression of a gene is not sufficient to make it a good vaccine candidate. The only way to determine if a protein is recognized by the immune system during latent infection with *M. tuberculosis* is to produce the given protein and test it in an appropriate assay as described herein. Of the more than 200 hundred antigens known to be expressed during primary infection, and tested as vaccines, less than a half dozen have demonstrated significant potential. So far only one antigen has been shown to have any potential as a therapeutic vaccine (Lowrie, 1999). However this vaccine only worked if given as a DNA vaccine, an experimental technique so far not approved for use in humans. Moreover, the technique has proved controversial, with other groups claiming that vaccination using this protocol induces either non-specific protection or even worsens disease (Turner, 2000).

What are needed are therapeutic vaccines that treat latent TB infection.

Summary of the Invention

The present invention provides therapeutic vaccines based on molecules that are induced or upregulated under the conditions of low oxygen transmission and restricted nutrients found in the granuloma (i.e., the location of latent TB infection). These vaccines are therapeutic and contrast with prior art vaccines which are designed to elicit protective immune responses prior to infection (prophylactic vaccination) that are only effective against primary infection. The immune responses elicited are powerless against the latent stage of the disease, because the bacteria have changed the antigens that they produce so that in essence they have altered their appearance and the immune system can no longer recognize them. However, latency is a dynamic process, maintained by the immune response, as indicated by the dramatic increase in the risk of reactivation of TB after HIV infection or other events that compromise immunity. Therefore, an effective vaccination strategy to protect infected individuals (therapeutic vaccination) is possible, but only if it is directed against those antigens expressed in the latent stage.

Further, the present invention provides a multiphase vaccine that combines components with prophylactic and therapeutic activity. In contrast, existing TB vaccines do not result in sterilizing immunity but rather control the infection at a subclinical level (thereby resulting in the subsequent establishment of latent infection. After conventional prophylactic vaccination, the evasion of the primary immune response and the subsequent development of latent disease are probably at least in part due to the change in the antigenic profile of the invading bacteria. Thus, vaccinating with antigens associated with latent TB prevents or reduces the establishment of latent infection and therefore, a vaccine incorporating antigens expressed by the bacteria both in the first logarithmic growth phase and during latent disease improve long-term immunity when used as a prophylactic vaccine. A multiphase vaccine of the invention will also be efficient as a therapeutic vaccine thereby addressing the problem that the majority of the population in the third world who would receive a future TB vaccine could be already latently infected.

For a number of years, a major effort has been put into the identification of protective antigens for the development of novel prophylactic vaccines against TB and today a few antigens with demonstrated protective activity in prophylactic vaccines have been identified (e.g. ESAT-6, the 38 kDa antigen, Ag85A and Ag85B). Such molecules are useful

components, which in combination with latency associated antigens such as α-crystalline, form a multiphase vaccine of the invention. Advantageously and in contrast to antigens in the art, the antigens described in the invention are incorporated in vaccines through the use of well-recognized vaccination technology, as demonstrated in provided examples.

Finally, the immunodominant antigens identified in this invention may be used as diagnostic reagents. Our group has abundantly demonstrated that antigens expressed by mycobacteria during the early stages of the infection, such as ESAT-6 (Early Secretory Antigen Target-6) are recognized in individuals who are in the process of developing primary TB, even though they are healthy at the time of diagnosis (Doherty 2002). However, the large numbers of contacts who are exposed, and almost certainly infected, remain negative to this antigen (Doherty 2002). Since those individuals latently infected remain healthy by making an immune response against the latent bacteria, they must be making an immune response to those antigens expressed by the latent bacteria. Thus, the antigens of the invention may also be used to diagnose latent infection and differentiate it from primary acute TB.

Other aspects and advantages of the invention will be readily apparent to one of skill in the art.

Brief Description of the Drawings

Figs. 1A and 1B illustrate the results of testing in TB vaccination models.
A schematic time schedule of the models for Fig. 1A, prophylactic vaccination and Fig. 1B, therapeutic vaccination. Each square on the time axis represents one week. Three prophylactic vaccinations two weeks apart are given 6 weeks prior to an aerosol infection. The protective effect of the vaccines is measured 6 weeks after infection, in the acute phase of the infection. For analysis of therapeutic vaccinations a reactivation model is established, where aerosol infected mice are treated with anti-*M tuberculosis* drugs for 8 weeks from the peak of infection (6 weeks after infection). This induces a latent infection phase with a low bacterial load. Four to five weeks into the latency phase three therapeutic vaccinations are given two weeks apart and the protective effect of the vaccines is measured as bacterial load in the reactivation phase, seven weeks after the last immunization.

Figs. 2A and 2B illustrate prophylactic and therapeutic vaccine induced protection. C57Bl/6j mice were immunized 3 times with a 2-week interval with recombinant ESAT6, BCG or recombinant Rv2031c. In Fig. 2A, the immunization was given as a prophylactic vaccine 6 weeks before the mice were given a *M. tuberculosis* infection (approx. 250 bacilli) through the aerosol route with. Bacterial numbers in the lung was enumerated 6 weeks post infection. In Fig. 2B, the immunization was given as a therapeutic vaccine after a latent infection had been established. Bacterial numbers in the lung was enumerated 8 weeks after the last immunization. The data represents the mean of 5 individual mice.

Fig. 3 illustrates Rv2031c specific IFN-γ responses. Latent infected C57Bl/6j mice were either not immunized or immunized with 3μg recombinant Rv2031 3 times with a two-week interval. One and two weeks post immunization mice were bleed and PBMCs isolated. The frequency of IFN-γ producing cells specific for either ESAT6 or Rv2031c was determined for both the rRv2031c immunized and the unimmunized group. In an ELIspot plate precoated with anti-IFN-γ antibodies graded numbers of PBMCs were incubated with either 2μg/ml rRv2031c or 2μg/ml rESAT6. After 32h the plate was washed and incubated with biotinylated anti-INF-γ antibodies followed by a streptavidin-alkalinephosphatase incubation. The INFγ spots, representing individual IFN-γ producing cells were visualized using BCIP/NBT substrate. The results are shown as number Rv2031c specific IFN-γ producing cell (black bars) and number of ESAT6 specific IFN-γ producing cell (hatch bars) per 10^6 PBMCs.

Figs. 4A and 4B illustrate the results of epitope screening of Rv2031c. PBMCs from rRv2031c immunized latently infected C57Bl/6j mice were analyzed for recognition of 20'mer overlapping peptides scanning through Rv2031c. In Fig. 4A, the peptides were analyzed in pools of 3-4 peptides. PBMCs ($2x10^5$) were incubated for 72 h with the peptide pools at 5 μg/ml per peptide. Supernatant was harvested and secreted IFN-γ was quantitated by ELISA. In Fig. 4B, individual peptides of positive pools were reanalyzed. PBMCs ($2x10^5$) were incubated for 72 h with 1 μg/ml of each peptide. Secreted IFN- γ in the supernatant was quantitated.

Figs. 5A and 5B illustrate protection against reactivation conferred by therapeutic vaccine given during latent infection. Latent infected C57Bl/6j mice were immunized 3 times with or without rRv2031c. Bacterial numbers in lung (Fig. 5A) and spleen (Fig. 5B) was enumerated 8 weeks after the last immunization. The data represents the mean of 8 individual mice.

Figs. 6A and 6B illustrate Rv0569 specific IFN-γ responses. Latent infected C57Bl/6j mice were vaccinated with 3μg of either recombinant Rv0569 or recombinant ESAT6 in a DDA/MPL adjuvant. The vaccines were given as 3 s.c. injections with a two-week interval and the induced immune response were evaluated 7 weeks after the last vaccination. Isolated splenocytes ($2x10^5$) were incubated for 72 h with antigen at 1 μg/ml. Supernatant was harvested and secreted IFN-γ was quantitated by ELISA using paired anti-murine IFN-γ antibodies (PharMingen) and recombinant IFN-γ (PharMingen) as standard. In Fig. 6A, Rv0569 specific response is measured in Rv0569-vaccinated and un-vaccinated latently infected mice; in Fig. 6B, the ESAT6 specific response is measured in ESAT6-vaccinated and un-vaccinated latently infected mice

Figs. 7A and 7B illustrate therapeutic vaccine induced protection against reactivation. Latently infected C57Bl/6j mice were vaccinated once with BCG or 3 times with a 2-week interval with either recombinant Rv0569 or recombinant ESAT6. Seven weeks after the last vaccination the bacterial numbers was enumerated in Fig. 7A, the lung and in Fig. 7B, the spleen of vaccinated and un-vaccinated mice. The data represents the mean of Log CFU per organ of 6-8 individual mice.

Detailed Description of the Invention

The invention is related to preventing, treating and detecting infections caused by species of the tuberculosis complex (*Mycobacterium tuberculosis*, *M. bovis*, *M. africanum*) by the use of a polypeptide comprising a *M. tuberculosis* antigen or an immunogenic portion or other variant thereof, or by the use of a DNA sequence encoding a *M. tuberculosis* antigen or an immunogenic portion or other variant thereof. The invention discloses a new therapeutic vaccine against tuberculosis comprising antigens induced during the latent stage of TB-infection. It also discloses a multiphase vaccine incorporating a combination of prophylactic and therapeutic antigens as well

as diagnostic reagents for the detection of the latent stage of *M. tuberculosis* infection.

The present invention discloses the use of one or more polypeptides, nucleic acids encoding these polypeptides or fragments hereof, which polypeptides are expressed during the latent stage of the mycobacteria infection, which stage is characterized by low-oxygen tension in the microenvironment of the mycobacteria, for a therapeutic vaccine against tuberculosis.

The polypeptides comprises one or more amino acid sequences selected from

(a) The sequences presented in Table 1.

(b) an immunogenic portion, e.g. a T-cell epitope, of any one of the sequences in (a); and /or

(c) an amino acid sequence analogue having at least 70% sequence identity to any one of the sequences in (a) or (b) and at the same time being immunogenic.

Table 1. Amino acid sequences of selected low oxygen induced antigens

Rv no.	SEQ ID NO:	Sequence
Rv0569	91	MKAKVGDWLVIKGATIDQPDHRGLIIEVRSSDGSPPYVVRWLETDHVATVIPGPDA VVVTAEEQNAADERAQHRFGAVQSAILHARGT
Rv0079	1	VEPKRSRLVVCAPEPSHAREFPDVAVFSGGRANASQAERLARAVGRVLADRGVTGG ARVRLTMANCADGPTLVQINLQVGDTPLRAQAATAGIDDLRPALIRLDRQIVRASA QWCPRPWPDRPRRRLTTPAEALVTRRKPVVLRRATPLQAIAAMDAMDYDVHLFTDA ETGEDAVVYRAGPSGLRLARQHHVFPPGWSRCRAPAGPPVPLIVNSRPTPVLTEAA AVDRAREHGLPFLFFTDQATGRGQLLYSRYDGNLGLITPTGDGVADGLA
Rv0080	2	MSPGSRRASPQSAREVVELDRDEAMRLLASVDHGRVVFTRAALPAIRPVNHLVVDG RVIGRTRLTAKVSVAVRSSADAGVVVAYEADDLDPRRRTGWSVVVTGLATEVSDPE QVARYQRLLHPWVNMAMDTVVAIEPEIVTGIRIVADSRTP
Rv0081	3	VESEPLYKLKAEFFKTLAHPARIRILELLVERDRSVGELLSSDVGLESSNLSQQLG VLRRAGVVAARRDGNAMIYSIAAPDIAELLAVARKVLARVLSDRVAVLEDLRAGG-SAT

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Rv no.	**SEQ ID NO:**	**Sequence**
Rv0363c	4	MPIATPEVYAEMLGQAKQNSYAFPAINCTSSETVNAAIKGFADAGSDGIIQF STGGAEFGSGLGVKDMVTGAVALAEFTHVIAAKYPVNVALHTDHCPKDKLDS YVRPLLAISAQRVSKGGNPLFQSHMWDGSAVPIDENLAIAQELLKAAAAAKI ILEIEIGVVGGEEDGVANEINEKLYTSPEDFEKTIEALGAGEHGKYLLAATF GNVHGVYKPGNVKLRPDILAQGQQVAAAKLGLPADAKPFDFVFHGGSGSLKS EIEEALRYGVVKMNVDTDTQYAFTRPIAGHMFTNYDGVLKVDGEVGVKKVYD PRSYLKKAEASMSQRVVQACNDLHCAGKSLTH
Rv0572c	5	MGEHAIKRHMRQRKPTKHPLAQKRGARILVFTDDPRRSVLIVPGCHLDSMRR EKNAYYFQDGNALVGMVVSGGTVEYDADDRTYVVQLTDGRHTTESSFEH- SSPSRSPQSDDL
Rv0574c	6	VAGNPDVVTVLLGGDVMLGRGVDQILPHPGKPQLRERYMRDATGYVRLAERV NGRIPLPVDWRWPWGEALAVLENTATDVCLINLETTITADGEFADRKPVCYR MHPDNVPALTALRPHVCALANNHILDFGYQGLTDTVAALAGAGIQSVGAGAD LLAARRSALVTVGHERRVIVGSVAAESSGVPESWAARRDRPGVWLIRDPAQR DVADDVAAQVLADKRPGDIAIVSMHWGSNWGYATAPGDVAFAHRLIDAGIDM VHGHSSHHPRPIEIYRGKPILYGCGDVVDDYEGIGGHESFRSELRLLYLTVT DPASGNLISLQMLPLRVSRMRLQRASQTDTEWLRNTIERISRRFGIRVVTRP DNLLEVVPAANLTSKE
Rv1264	7	VTDHVREADDANIDDLLGDLGGTARAERAKLVEWLLEQGITPDEIRATNPPL LLATRHLVGDDGTYVSAREISENYGVDLELLQRVQRAVGLARVDDPDAVVHM RADGEAAARAQRFVELGLNPDQVVLVVRVLAEGLSHAAEAMRYTALEAIMRP GATELDIAKGSQALVSQIVPLLGPMIQDMLFMQLRHMMETEAVNAGERAAGK PLPGARQVTVAFADLVGFTQLGEVVSAEELGHLAGRLAGLARDLTAPPVWFI KTIGDAVMLVCPDPAPLLDTVLKLVEVVDTDNNFPRLRAGVASGMAVSRAGD WFGSPVNVASRVTGVARPGAVLVADSVREALGDAPEADGFQWSFAGPRRLRG IRGDVRLFRVRRGATRTGSGGAAQDDDLAGSSP
Rv1592c	8	MVEPGNLAGATGAEWIGRPPHEELQRKVRPLLPSDDPFYFPPAGYQHAVPGT VLRSRDVELAFMGLIPQPVTATQLLYRTTNMYGNPEATVTTVIVPAELAPGQ TCPLLSYQCAIDAMSSRCFPSYALRRRAKALGSLTQMELLMISAALAEGWAV SVPDHEGPKGLWGSPYEPGYRVLDGIRAALNSERVGLSPATPIGLWGYSGGG LASAWAAEACGEYAPDLDIVGAVLGSPVGDLGHTFRRLNGTLLAGLPALVVA ALQHSYPGLARVIKEHANDEGRQLLEQLTEMTTVDAVIRMAGRDMGDFLDEP LEDILSTPEISHVFGDTKLGSAVPTPPVLIVQAVHDYLIDVSDIDALADSYT AGGANVTYHRDLFSEHVSLHPLSAPMTLRWLTDRFAGKPLTDHRVRTTWPTI FNPMTYAGMARLAVIAAKVITGRKLSRRPL
Rv1733c	9	MIATTRDREGATMITFRLRLPCRTILRVFSRNPLVRGTDRLEAVVMLLAVTV SLLTIPFAAAAGTAVQDSRSHVYAHQAQTRHPATATVIDHEGVIDSNTTATS APPRTKITVPARWVVNGIERSGEVNAKPGTKSGDRVGIWVDSAGQLVDEPAP PARAIADAALAALGLWLSVAAVAGALLALTRAILIRVRNASWQHDIDSLFC- TQR
Rv1734c	10	MTNVGDQGVDAVFGVIYPPQVALVSFGKPAQRVCAVDGAIHVMTTVLATLPA DHGCSDDHRGALFFLSINELTRCAAVTG

Table 1. (continued) Amino acid sequences of selected low oxygen induced antigens		
Rv no.	**SEQ ID NO:**	**Sequence**
Rv1736c	11	VTVTPRTGSRIEELLARSGRFFIPGEISADLRTVTRRGGRDGDVFYRDRWSHDK VVRSTHGVNCTGSCSWKIYVKDDIITWETQETDYPSVGPDRPEYEPRGCPRGAA FSWYTYSPTRVRHPYARGVLVEMYREAKARLGDPVAAWADIQADPRRRRRYQRA RGKGGLVRVSWAEATEMIAAAHVHTISTYGPDRVAGFSPIPAMSMVSHAAGSRF VELIGGVMTSFYDWYADLPVASPQVFGDQTDVPESGDWWDVVWQCASVLLTYPN SRQLGTAEELLAHIDGPAADLLGRTVSELRRADPLTAATRYVDTFDLRGRATLY LTYWTAGDTRNRGREMLAFAQTYRSTDVAPPRGETPDFLPVVLEFAATVDPEAG RRLLSGYRVPIAALCNALTEAALPYAHTVAAVCRTGDMMGELFWTVVPYVTMTI VAVGSWWRYRYDKFGWTTRSSQLYESRLLRIASPMFHFGILVVIVGHGIGLVIP QSWTQAAGLSEGAYHVQAVVLGSIAGITTLAGVTLLIYRRRTRGPVFMATTVND KVMYLVLVAAIVAGLGATALGSGVVGEAYNYRETVSVWFRSVWVLQPRGDLMAE APLYYQIHVLIGLALFALWPFTRLVHAFSAPIGYLFRPYIIYRSREELVLTR- PRRRGW
Rv1737c	12	MRGQAANLVLATWISVVNFWAWNLIGPLSTSYARDMSLSSAEASLLVATPILVG ALGRIVTGPLTDRFGGRAMLIAVTLASILPVLAVGVAATMGSYALLVFFGLFLG VAGTIFAVGIPFANNWYQPARRGFSTGVFGMGMVGTALSAFFTPRFVRWFGLFT THAIVAAALASTAVVAMVVLRDAPYFRPNADPVLPRLKAAARLPVTWEMSFLYA IVFGGFVAFSNYLPTYITTIYGFSTVDAGARTAGFALAAVLARPVGGWLSDRIA PRHVVLASLAGTALLAFAAALQPPPEVWSAATFITLAVCLGVGTGGVFAWVARR APAASVGSVTGIVAAAGGLGGYFPPLVMGATYDPVDNDYTVGLLLLVATALVAC TYTALHAREPVSEEASR
Rv1738c	13	MCGDQSDHVLQHWTVDISIDEHEGLTRAKARLRWREKELVGVGLARLNPADRNV PEIGDELSVARALSDLGKRMLKVSTHDIEAVTHQPARLLY
Rv1739c	14	MIPTMTSAGWAPGVVQFREYQRRWLRGDVLAGLTVAAYLIPQAMAYATVAGLPP AAGLWASIAPLAIYALLGSSRQLSIGPESATALMTAAVLAPMAAGDLRRYAVLA ATLGLLVGLICLLAGTARLGFLASLRSRPVLVGYMAGIALVMISSQLGTITGTS VEGNEFFSEVHSFATSVTRVHWPTFVLAMSVLALLTMLTRWAPRAPGPIIAVLA ATMLVAVMSLDAKGIAIVGRIPSGLPTPGVPPVSVEDLRALIIPAAGIAIVTFT DGVLTARAFAARRGQEVNANAELRAVGACNIAAGLTHGFPVSSSSSRTALADVV GGRTQLYSLIALGLVVIVMVFASGLLAMFPIAALGALVVYAALRLIDLSEFRRL ARFRRSELMLALATTAAVLGLGVFYGVLAAVALSILELLRRVAHPHDSVLGFVP GIAGMHDIDDYPQAKRVPGLVVYRYDAPLCFANAEDFRRRALTVVDQDPGQVEW FVLNAESNVEVDLTALDALDQLRTELLRRGIVFAMARVKQDLRESLRAASLLDK IGEDHIFMTLPTAVQAFRRR
Rv1813c	15	MITNLRRRTAMAAAGLGAALGLGILLVPTVDAHLANGSMSEVMMSEIAG LPIPPIIHYGAIAYAPSGASGKAWHQRTPARAEQVALEKCGDKTCKVVS RFTRCGAVAYNGSKYQGGTGLTRRAAEDDAVNRLEGGRIVNWACN

Table 1. (continued) Amino acid sequences of selected low oxygen induced antigens		
Rv no.	**SEQ ID NO:**	**Sequence**
Rv1997c	16	LSASVSATTAHHGLPAHEVVLLLESDPYHGLSDGEAAQRLERFGPNTLAVVTRASL LARILRQFHHPLIYVLLVAGTITAGLKEFVDAAVIFGVVVINAIVGFIQESKAEAA LQGLRSMVHTHAKVVREGHEHTMPSEELVPGDLVLLAAGDKVPADLRLVRQTGLSV NESALTGESTPVHKDEVALPEGTPVADRRNIAYSGTLVTAGHGAGIVVATGAETEL GEIHRLVGAAEVVATPLTAKLAWFSKFLTIAILGLAALTFGVGLLRRQDAVETFTA AIALAVGAIPEGLPTAVTITLAIGMARMAKRRAVIRRLPAVETLGSTTVICADKTG TLTENQMTVQSIWTPHGEIRATGTGYAPDVLLCDTDDAPVPVNANAALRWSLLAGA CSNDAALVRDGTRWQIVGDPTEGAMLVVAAKAGFNPERLATTLPQVAAIPFSSERQ YMATLHRDGTDHVVLAKGAVERMLDLCGTEMGADGALRPLDRATVLRATEMLTSRG LRVLATGMGAGAGTPDDFDENVIPGSLALTGLQAMSDPPRAAAASAVAACHSAGIA VKMITGDHAGTATAIATEVGLLDNTEPAAGSVLTGAELAALSADQYPEAVDTASVF ARVSPEQKLRLVQALQARGHVVAMTGDGVNDAPALRQANIGVAMGRGGTEVAKDAA DMVLTDDDFATIEAAVEEGRGVFDNLTKFITWTLPTNLGEGLVILAAIAVGVALPI LPTQILWINMTTAIALGLMLAFEPKEAGIMTRPPRDPDQPLLTGWLVRRTLLVSTL LVASAWWLFAWELDNGAGLHEARTAALNLFVVVEAFYLFSCRSLTRSAWRLGMFAN RWIILGVSAQAIAQFAITYLPAMNMVFDTAPIDIGVWVRIFAVATAITIVVATDTL LPRIRAQPP
Rv1998c	17	MSFHDLHHQGVPFVLPNAWDVPSALAYLAEGFTAIGTTSFGVSSSGGHPDGHRATR GANIALAAALAPLQCYVSVDIEDGYSDEPDAIADYVAQLSTAGINIEDSSAEKLID PALAAAKIVAIKQRNPEVFVNARVDTYWLRQHADTTSTIQRALRYVDAGADGVFVP LANDPDELAELTRNIPCPVNTLPVPGLTIADLGELGVARVSTGSVPYSAGLYAAAH AARAVSDGEQLPRSVPYAELQARLVDYENRTSTT
Rv2003c	18	VVKRSRATRLSPSIWSGWESPQCRSIRARLLLPRGRSRPPNADCCWNQLAVTPDTR MPASSAAGRDAAAYDAWYDSPTGRPILATEVAALRPLIEVFAQPRLEIGVGTGRFA DLLGVRFGLDPSRDALMFARRRGVLVANAVGEAVPFVSRHFGAVLMAFTLCFVTDP AAIFRETRRLLADGGGLVIGFLPRGTPWADLYALRAARGQPGYRDARFYTAAELEQ LLADSGFRVIARRCTLHQPPGLARYDIEAAHDGIQAGAGFVAISAVDQAHEPKD- DHPLESE
Rv2005c	19	MSKPRKQHGVVVGVDGSLESDAAACWGATDAAMRNIPLTVVHVVNADVATWPPMPY PETWGVWQEDEGRQIVANAVKLAKEAVGADRKLSVKSELVFSTPVPTMVEISNEAE MVVLGSSGRGALARGLLGSVSSSLVRRAGCPVAVIHSDDAVIPDPQHAPVLVGIDG SPVSELATAVAFDEASRRGVELIAVHAWSDVEVVELPGLDFSAVQQEAELSLAERL AGWQERYPDVPVSRVVVCDRPARKLVQKSASAQLVVVGSHGRGGLTGMLLGSVSNA VLHAARVPVIVARQS
Rv2007c	20	VTYVIGSECVDVMDKSCVQECPVDCIYEGARMLYINPDECVDCGACKPACRVEAIY WEGDLPDDQHQHLGDNAAFFHQVLPGRVAPLGSPGGAAAVGPIGVDTPLVAAIP- VECP
Rv2028c	21	MNQSHKPPSIVVGIDGSKPAVQAALWAVDEAASRDIPLRLLYAIEPDDPGYAAHGA AARKLAAAENAVRYAFTAVEAADRPVKVEVEITQERPVTSLIRASAAAALVCVGAI GVHHFRPERVGSTAAALALSAQCPVAIVRPHRVPIGRDAAWIVVEADGSSDIGVLL GAVMAEARLRDSPVRVVTCRQSGVGDTGDDVRASLDRWLARWQPRYPDVRVQSAAV HGELLDYLAGLGRSVHMVVLSASDQEHVEQLVGAPGNAVLQEAGCTLLVVGQQYL
Rv2029c	22	MTEPAAWDEGKPRIITLTMNPALDITTSVDVVRPTEKMRCGAPRYDPGGGGINVAR IVHVLGGCSTALFPAGGSTGSLLMALLGDAGVPFRVIPIAASTRESFTVNESRTAK QYRFVLPGPSLTVAEQEQCLDELRGAAASAAFVVASGSLPPGVAADYYQRVADICR RSSTPLILDTSGGGLQHISSGVFLLKASVRELRECVGSELLTEPEQLAAAHELIDR GRAEVVVVSLGSQGALLATRHASHRFSSIPMTAVSGVGAGDAMVAAITVGLSRGWS LIKSVRLGNAAGAAMLLTPGTAACNRDDVERFFELAAEPTEVGQDQYVWHPIVN- PEASP

Table 1. (continued) Amino acid sequences of selected low oxygen induced antigens

Rv no.	SEQ ID NO:	Sequence
Rv2030c	23	VLMTAAADVTRRSPRRVFRDRREAGRVLAELLAAYRDQPDVIVLGLARGGLPVAWE VAAALHAPLDAFVVRKLGAPGHDEFAVGALASGGRVVVNDDVVRGLRITPQQLRDI AEREGRELLRRESAYRGERPPTDITGKTVIVVDDGLATGASMFAAVQALRDAQPAQ IVIAVPAAPESTCREFAGLVDDVVCATMPTPFLAVGESFWDFRQVTDEEVRRLLAT PTAGPSLRRPAASTAADVLRRVAIDAPGGVPTHEVLAELVGDARIVLIGESSHGTH EFYQARAAMTQWLIEEKGFGAVAAEADWPDAYRVNRYVRGLGEDTNADEALSGFER FPAWMWRNTVVRDFVEWLRTRNQRYESGALRQAGFYGLDLYSLHRSIQEVISYLDK VDPRAAARARARYACFDHACADDGQAYGFAAAFGAGPSCEREAVEQLVDVQRNALA YARQDGLLAEDELFYAQQNAQTVRDAEVYYRAMFSGRVTSWNLRDQHMAQTLGSLL THLDRHLDAPPARIVVWAHNSHVGDARATEVWADGQLTLGQIVRERYGDESRSIGF STYTGTVTAASEWGGIAQRKAVRPALHGSVEELFHQTADSFLVSARLSRDAEAPLD VVRLGRAIGVVYLPATERQSHYLHVRPADQFDAMIHIDQTRALEPLEVTSRWIAGE NPETYPTGL
Rv2031c	24	MATTLPVQRHPRSLFPEFSELFAAFPSFAGLRPTFDTRLMRLEDEMKEGRYEVRAE LPGVDPDKDVDIMVRDGQLTIKAERTEQKDFDGRSEFAYGSFVRTVSLPVGA- DEDDIKATYDKGILTVSVAVSEGKPTEKHIQIRSTN
Rv2032	25	MPDTMVTTDVIKSAVQLACRAPSLHNSQPWRWIAEDHTVALFLDKDRVLYATDHSG REALLGCGAVLDHFRVAMAAAGTTANVERFPNPNDPLHLASIDFSPADFVTEGHRL RADAILLRRTDRLPFAEPPDWDLVESQLRTTVTADTVRIDVIADDMRPELAAASKL TESLRLYDSSYHAELFWWTGAFETSEGIPHSSLVSAAESDRVTFGRDFPVVANTDR RPEFGHDRSKVLVLSTYDNERASLLRCGEMLSAVLLDATMAGLATCTLTHITELHA SRDLVAALIGQPATPQALVRVGLAPEMEEPPPATPRRPIDEVFHVRAKDHR
Rv2428	26	MPLLTIGDQFPAYQLTALIGGDLSKVDAKQPGDYFTTITSDEHPGKWRVVFFWPKD FTFVCPTEIAAFSKLNDEFEDRDAQILGVSIDSEFAHFQWRAQHNDLKTLPFPMLS DIKRELSQAAGVLNADGVADRVTFIVDPNNEIQFV- SATAGSVGRNVDEVLRVLDALQSDELCACNWRKGDPTLDAGELLKASA
Rv2624c	27	MSGRGEPTMKTIIVGIDGSHAAITAALWGVDEAISRAVPLRLVSVIKPTHPSPDDY DRDLAHAERSLREAQSAVEAAGKLVKIETDIPRGPAGPVLVEASRDAEMICVGSVG IGRYASSILGSTATELAEKAHCPVAVMRSKVDQPASDINWIVVRMTDAPDNEAVLE YAAREAKLRQAPILALGGRPEELREIPDGEFERRVQDWHHRHPDVRVYPITTHTGI ARFLADHDERVQLAVIGGGEAGQLARLVGPSGHPVFRHAECSVLVVRR
Rv2625c	28	MRDAIPLGRIAGFVVNVHWSVLVILWLFTWSLATMLPGTVGGYPAVVYWLLGAGGA VMLLASLLAHELAHAVVARRAGVSVESVTLWLFGGVTALGGEAKTPKAAFRIAFAG PATSLALSATFGALAITLAGVRTPAIVISVAWWLATVNLLLGLFNLLPGAPLDGGR LVRAYLWRRHGDSVRAGIGAARAGRVVALVLIALGLAEFVAGGLVGGVWLAFIGWF IFAAAREEETRISTQQLFAGVRVADAMTAQPHTAPGWINVEDFIQRYVLGERHSAY PVADRDGSITGLVALRQLRDVAPSRRSTTSVGDIALPLHSVPTARPQEPLTALLER MAPLGPRSRALVTEGSAVVGIVTPSDVARLIDVYRLAQPEPTFTTSPQDADRFS- DAG
Rv2727c	29	MASSASDGTHERSAFRLSPPVLSGAMGPFMHTGLYVAQSWRDYLGQQPDKLPIARP TIALAAQAFRDEIVLLGLKARRPVSNHRVFERISQEVAAGLEFYGNRRWLEKPSGF FAQPPPLTEVAVRKVKDRRRSFYRIFFDSGFTPHPGEPGSQRWLSYTANNREYALL LRHPEPRPWLVCVHGTEMGRAPLDLAVFRAWKLHDELGLNIVMPVLPMHGPRGQGL PKGAVFPGEDVLDDVHGTAQAVWDIRRLLSWIRSQEEESLIGLNGLSLGGYIASLV ASLEEGLACAILGVPVADLIELLGRHCGLRHKDPRRHTVKMAEPIGRMISPLSLTP LVPMPGRFIYAGIADRLVHPREQVTRLWEHWGKPEIVWYPGGHTGFFQSRPVRRFV QAALEQSGLLDAPRTQRDRSA

Table 1. (continued) Amino acid sequences of selected low oxygen induced antigens		
Rv no.	**SEQ ID No**	**Sequence**
Rv2628	30	MSTQRPRHSGIRAVGPYAWAGRCGRIGRWGVHQEAMMNLAIWHPRKVQSATIYQVT DRSHDGRTARVPGDEITSTVSGWLSELGTQSPLADELARAVRIGDWPAAYAIGEHL SVEIAVAV
Rv2629	31	MRSERLRWLVAAEGPFASVYFDDSHDTLDAVERREATWRDVRKHLESRDAKQELID SLEEAVRDSRPAVGQRGRALIATGEQVLVNEHLIGPPPATVIRLSDYPYVVPLIDL EMRRPTYVFAAVDHTGADVKLYQGATISSTKIDGVGYPVHKPVTAGWNGYGDFQHT TEEAIRMNCRAVADHLTRLVDAADPEVVFVSGEVRSRTDLLSTLPQRVAVRVSQLH AGPRKSALDEEEIWDLTSAEFTRRRYAEITNVAQQFEAEIGRGSGLAAQGLAEVCA ALRDGDVDTLIVGELGEATVVTGKARTTVARDADMLSELGEPVDRVARADEALPFA AIAVGAALVRDDNRIAPLDGVGALLRYAATNRLGSHRS
Rv2630	32	MLHRDDHINPPRPRGLDVPCARLRATNPLRALARCVQAGKPGTSSGHRSVPHTADL RIEAWAPTRDGCIRQAVLGTVESFLDLESAHAVHTRLRRLTADRDDDLLVAVLEEV IYLLDTVGETPVDLRLRDVDGGVDVTFATTDASTLVQVGAVPKAVSLNELRFSQGR HGWRCAVTLDV
Rv2659c	33	VTQTGKRQRRKFGRIRQFNSGRWQASYTGPDGRVYIAPKTFNAKIDAEAWLTDRRR EIDRQLWSPASGQEDRPGAPFGEYAEGWLKQRGIKDRTRAHYRKLLDNHILATFAD TDLRDITPAAVRRWYATTAVGTPTMRAHSYSLLRAIMQTALADDLIDSNPCRISGA STARRVHKIRPATLDELETITKAMPDPYQAFVLMAAWLAMRYGELTELRRKDIDLH GEVARVRRAVVRVGEGFKVTTPKSDAGVRDISIPPHLIPAIEDHLHKHVNPGRESL LFPSVNDPNRHLAPSALYRMFYKARKAAGRPDLRVHDLRHSGAVLAASTGATLAEL MQRLGHSTAGAALRYQHAAKGRDREIAALLSKLAENQEM
Rv2780	34	MRVGIPTETKNNEFRVAITPAGVAELTRRGHEVLIQAGAGEGSAITDADFKAAGAQ LVGTADQVWADADLLLKVKEPIAAEYGRLRHGQILFTFLHLAASRACTDALLDSGT TSIAYETVQTADGALPLLAPMSEVAGRLAAQVGAYHLMRTQGGRGVLMGGVPGVEP ADVVVIGAGTAGYNAARIANGMGATVTVLDINIDKLRQLDAEFCGRIHTRYSSAYE LEGAVKRADLVIGAVLVPGAKAPKLVSNSLVAHMKPGAVLVDIAIDQGGCFEGSRP TTYDHPTFAVHDTLFYCVANMPASVPKTSTYALTNATMPYVLELADHGWRAACRSN PALAKGLSTHEGALLSERVATDLGVPFTEPASVLA
Rv3126c	35	MVIRFDQIGSLVLSMKSLASLSFQRCLRENSSLVAALDRLDAAVDELSALSFDALT TPERDRARRDRDHHPWSRSRSQLSPRMAHGAVHQCQWPKAVWAVIDNP
Rv3127	36	VLKNAVLLACRAPSVHNSQPWRWVAESGSEHTTVHLFVNRHRTVPATDHSGRQAII SCGAVLDHLRIAMTAAHWQANITRFPQPNQPDQLATVEFSPIDHVTAGQRNRAQAI LQRRTDRLPFDSPMYWHLFEPALRDAVDKDVAMLDVVSDDQRTRLVVASQLSEVLR RDDPYYHAELEWWTSPFVLAHGVPPDTLASDAERLRVDLGRDFPVRSYQNRRAELA DDRSKVLVLSTPSDTRADALRCGEVLSTILLECTMAGMATCTLTHLIESSDSRDIV RGLTRQRGEPQALIRVGIAPPLAAVPAPTPRRPLDSVLQIRQTPEKGRNASDRNAR ETGWFSPP
Rv3128c	37	VWSASGGQCGKYLAASMVLQLDGLERHGVLEFGRDRYGPEVREELLAMSAASIDRY LKTAKAKDQISGVSTTKPSPLLRNSIKVRRAGDEVEAEPGFFEGDTVAHCGPTLKG EFAHTLNLTDVHIGWVFTRTVRNNARTHILAGLKASVTEIPHGITGLDFDNGTVFL NKPVISWAGDNGI- YFTRFRPYKKNH*ATIESKNNHLVRKYAFYYRYDTAEERAVLNRMWKLVNDRLNYL TPTIKPIGYASSADGRRRRLYDAPQTPLDRPLAARVLSAAQQADLITYRDSLNPAQ IGRKIADLQNRLLILAKEKTEQLYLANIPTALPDIHKGILIKAG
Rv3129	38	VVQGRTVLFRTAEGAKLFSAVAKCAVAFEADDHNVAEGWSVIVKVRAQVLTTDAGV REAERAQLLPWTATLKRHCVRVIPWEITGRHFRFGPEPDRSQTFACEASSHNQR

Table 1. (continued) Amino acid sequences of selected low oxygen induced antigens

Rv no.	SEQ ID No	Sequence
Rv3130c	39	MNHLTTLDAGFLKAEDVDRHVSLAIGALAVIEGPAPDQEAFLSSLAQRLRPCTRFG QRLRLRPFDLGAPKWVDDPDFDLGRHVWRIALPRPGNEDQLFELIADLMARRLDRG RPLWEVWVIEGLADSKWAILTKLHHCMADGIAATHLLAGLSDESMSDSFASNIHTT MQSQSASVRRGGFRVNPSEALTASTAVMAGIVRAAKGASEIAAGVLSPAASSLNGP ISDLRRYSAAKVPLADVEQVCRKFDVTINDVALAAITESYRNVLIQRGERPRFDSL RTLVPVSTRSNSALSKTDNRVSLMLPNLPVDQENPLQRLRIVHSRLTRAKAGGQRQ FGNTLMAIANRLPFPMTAWAVGLLMRLPQRGVVTVATNVPGPRRPLQIMGRRVLDL YPVSPIAMQLRTSVAMLSYADDLYFGILADYDVVADAGQLARGIEDAVARLVAISK RRKVTRRRGALSLVV
Rv3131	40	MNTHFPDAETVRTVLTLAVRAPSIHNTQPWRWRVCPTSLELFSRPDMQLRSTDPDG RELILSCGVALHHCVVALASLGWQAKVNRFPDPKDRCHLATIGVQPLVPDQADVAL AAAIPRRRTDRRAYSCWPVPGGDIALMAARAARGGVMLRQVSALDRMKAIVAQAVL DHVTDEEYLRELTIWSGRYGSVAGVPARNEPPSDPSAPIPGRLFAGPGLSQPSDVL PADDGAAILALGTETDDRLARLRAGEAASIVLLTATAMGLACCPITEPLEIAKTRD AVRAEVFGAGGYPQMLLRVGWAPINADPLPPTPRRELSQVVEWPEELLRQRC
Rv3132	41	MTTGGLVDENDGAAMRPLRHTLSQLRLHELLVEVQDRVEQIVEGRDRLDGLVEAML VVTAGLDLEATLRAIVHSATSLVDARYGAMEVHDRQHRVLHFVYEGIDEETVRRIG HLPKGLGVIGLLIEDPKPLRLDDVSAHPASIGFPPYHPPMRTFLGVPVRVRDESFG TLYLTDKTNGQPFSDDDEVLVQALAAAAGIAVANARLYQQAKARQSWIEATRDIAT ELLSGTEPATVFRLVAAEALKLTAADAALVAVPVDEDMPAADVGELLVIETVGSAV ASIVGRTIPVAGAVLREVFVNGIPRRVDRVDLEGLDELADAGPALLLPLRARGTVA GVVVVLSQGGPGAFTDEQLEMMAAFADQAALAWQLATSQRRMRELDVLTDRDRIAR DLHDHVIQRLFAIGLALQGAVPHERNPEVQQRLSDVVDDLQDVIQEIRTTIYDLHG ASQGITRLRQRIDAAVAQFADSGLRTSVQFVGPLSVVDSALADQAEAVVREAVSNA VRHAKASTLTVRVKVDDDLCIEVTDNGRGLPDEFTGSGLTNLRQRAEQAGGEFTLA SVPGASGTVLRWSAPLSQ
Rv3134c	42	MSDPRPARAVVVGIDGSRAATHAALWAVDEAVNRDIPLRLVYVIDPSQLSAAGEGG GQSAARAALHDASRKVEATGQPVKIETEVLCGRPLTKLMQESRSAAMLCVGSVGLD HVRGRRGSVAATLAGSALCPVAVIHPSPAEPATTSQVSAVVAEVDNGVVLRHAFEE ARLRGVPLRAVAVHAAETPDDVEQGSRLAHVHLSRRLAHWTRLYPEVRVDRAIAGG SACRHLAANAKPGQLFVADSHSAHELCGAYQPGCAVLTVRSANL
Rv3841	43	MTEYEGPKTKFHALMQEQIHNEFTAAQQYVAIAVYFDSEDLPQLAKHFYSQAVEER NHAMMLVQHLLDRDLRVEIPGVDTVRNQFDRPREALALALDQERTVTDQVGRLTAV ARDEGDFLGEQFMQWFLQEQIEEVALMATLVRVADRAGANLFELENFVAREVDVAP AASGAPHAAGGRL
Rv3842c	44	MTWADEVLAGHPFVVAHRGASAARPEHTLAAYDLALKEGADGVECDVRLTRDGHL VCVHDRRLDRTSTGAGLVSTMTLAQLRELEYGAWHDSWRPDGSHGDTSLLTLDAL VSLVLDWHRPVKIFVETKHPVRYGSLVENKLLALLHRFGIAAPASADRSRAVVMS FSAAAVWRIRRAAPLLPTVLLGKTPRYLTSSAATAVGATAVGPSLPALKEYPQLV DRSAAQGRAVYCWNVDEYEDIDFCREVGVAWIGTHHPGRTKAWLEDGRANGTTR
Rv3908	45	VSDGEQAKSRRRRGRRRGRRAAATAENHMDAQPAGDATPTPATAKRSRSRSPRRG STRMRTVHETSAGGLVIDGIDGPRDAQVAALIGRVDRRGRLLWSLPKGHIELGET AEQTAIREVAEETGIRGSVLAALGRIDYWFVTDGRRVHKTVHHYLMRFLGGELSD EDLEVAEVAWVPIRELPSRLAYADERRLAEVADE- LIDKLQSDGPAALPPLPPSSPRRRPQTHSRARHADDSAPGQHNGPGPGP

Table 2. DNA sequences of selected low oxygen induced antigens		
Rv no.	**SEQ ID NO:**	**Sequence**
Rv0079	**46**	Gtggaaccgaaacgcagtcgcctcgtcgtatgtgcacccgagccatcgcacgcgcg ggaattcccggatgtcgccgtattctccggcggccgggctaacgcatcccaggccg aacggttggctcgtgccgtgggtcgcgtgttggccgatcggggcgtcaccgggggt gctcgggtgcggctgaccatggcgaactgcgccgatgggccgacgctggtgcagat aaacctgcaggtaggtgacaccccattaagggcgcaggccgccaccgcgggcatcg atgatctgcgacccgcactgatcagactggatcgacagatcgtgcgggcgtcggca cagtggtgcccccggccttggccggatcggccccgccggcgattgaccacgccggc cgaggcgctagtcacccgccgcaaaccggtcgtgctaaggcgcgcaaccccgttgc aggcgattgccgctatggacgccatggactacgacgtgcatttgttcaccgacgcc gagacgggggaggacgctgtggtctatcgggctggaccgtcggggctgcggctggc ccgccagcaccacgtatttcccccaggatggtcacgttgtcgcgccccagccgggc cgccggtgccgctgattgtgaattcgcgtccgacaccggttctcacggaggccgcc gcggtggaccgggcgcgcgaacatggactgccattcctgtttttcaccgaccaggc caccggccgcggccagctgctctactcccgctacgacggcaacctcgggttgatca ccccgaccggtgacggcgttgccgacggtctggca
Rv0080	**47**	Atgagcccgggctcgcggcgcgccagcccgcaaagcgcccgggaggtggtcgagct cgaccgtgacgaggcgatgcggttgctggccagcgttgaccatgggcgtgtggtgt tcacccgcgcggcgctgccggcgatccgtccagtcaatcacctcgtggtcgacggt cgggtgatcgggcgcacccgcctgacggccaaggtgtccgttgcggtgcgatcgag cgccgatgccggtgtcgtggtcgcctacgaagccgacgaccttgatccgcggcgtc ggacggggtggagtgtggtggtgacgggactggcgaccgaggtcagcgatcccgag caggttgcccgctaccagcggctgctacacccgtgggtgaacatggcgatggacac cgtggtcgcgatcgaacccgagatcgtcaccggcatccgcatcgttgctgactcgc gtacgccg
Rv0081	**48**	gtggagtccgaaccgctgtacaagctcaaggcggagttcttcaaaaccccttgcgca tccggcgcggatcaggattttggagctgctggtcgagcgggaccgttcggtcggtg agttgctgtcctcggacgtcggcctggagtcgtcgaacctgtcccagcagctgggt gtgctacgccgggcgggtgttgtcgcggcacgtcgtgacggcaacgcgatgatcta ttcgattgccgcacccgatatcgccgagctgctggcggtggcacgcaaggtgctgg ccagggtgctcagcgaccgggtggcggtgctagaggacctccgcgccggcggc- tcggccacg
Rv0363c	**49**	Atgcctatcgcaacgcccgaggtctacgcggagatgctcggtcaggccaaacaaaa ctcgtacgctttcccggctatcaactgcacctcctcggaaaccgtcaacgccgcga tcaaaggtttcgccgacgccggcagtgacggaatcatccagttctcgaccggtggc gcagaattcggctccggcctcggggtcaaagacatggtgaccggtgcggtcgcctt ggcggagttcacccacgttatcgcggccaagtacccggtcaacgtggcgctgcaca ccgaccactgccccaaggacaagttggacagctatgtccggcccttgctggcgatc tcggcgcaacgcgtgagcaaaggtggcaatcctttgttccagtcgcacatgtggga cggctcggcagtgccaatcgatgagaacctggccatcgcccaggagctgctcaagg cggcggcggccgccaagatcattctggagatcgagatcggcgtcgtcggcggcgaa gaggacggcgtggcgaacgagatcaacgagaagctgtacaccagcccggaggactt cgagaaaaccatcgaggcgctgggcgccggtgagcacggcaaatacctgctggccg cgacgttcggcaacgtgcatggcgtctacaagcccggcaacgtcaagcttcgcccc gacatccttgcgcaagggcaacaggtggcggcggccaagctcggactgccggccga cgccaagccgttcgacttcgtgttccacggcggctcgggttcgcttaagtcggaga tcgaggaggcgctgcgctacggcgtggtgaagatgaacgtcgacaccgacacccag tacgcgttcacccgcccgatcgccggtcacatgttcaccaactacgacggagtgct caaggtcgatggcgaggtgggtgtcaagaaggtctacgacccgcgcagctacctca agaaggccgaagcttcgatgagccagcgggtcgttcaggcgtgcaatgacctgcac tgcgccggaaagtccctaacccac

Table 2. (continued) DNA sequences of selected low oxygen induced antigens		
Rv no.	**SEQ ID NO:**	**Sequence**
Rv0572c	**50**	atgggtgagcacgccatcaagcggcacatgcggcaacggaagcctacgaagcatcc cctagcccagaaacggggcgcgcggattctggtcttcaccgacgatccccgcagga gcgtcctcatagtgcccggttgccacctggattccatgcgccgagaaaagaacgcg tactacttccaggacggcaatgcgttggttgggatggttgtctcgggcggcacggt tgagtacgacgccgacgaccgcacatatgtcgtgcagctcaccgacggaaggcaca ccactgagtcatctttcgaacactcatcgccgagtcgatcacctcaatccgat- gaccta
Rv0574c	**51**	gtggctggcaatcctgatgtggtgacggtgctgctgggcggtgacgtcatgctcgg ccgtggcgtcgatcagatcctgcctcatcccggcaaaccgcaattgcgcgaacggt atatgcgggatgcgaccggctatgttcgcctggccgagcgggtgaacgggcgcatt ccgctccccgtggattggcgctggccctggggcgaggcgttggcggtccttgagaa caccgcgaccgacgtctgtttgatcaatctggagacgacgatcaccgccgacggtg aattcgccgaccgcaaaccggtctgctaccggatgcacccggataacgtgccggcg ctgacggcattgcggccgcacgtgtgcgcgctggccaacaaccacattctcgattt cggctaccaggggctgaccgatacggtcgcggctctcgccggtgcggggatccaga gtgtcggggcgggagccgatttgctcgccgctcgccgctcggcgctagtcacggtt ggccatgaacgccgggtgatcgtcggctcggtagcggcggaatccagcggcgtccc cgaatcctgggccgcccgccgcgaccggcccggagtgtggttgatccgggatccgg cgcaacgcgacgtcgccgacgatgtggcggcacaggtgctggcggacaaacgcccc ggcgatatcgccatagtctcgatgcattggggatccaattggggctatgcgaccgc acccggcgacgtcgcgttcgcgcaccgactgatcgacgccggcatcgacatggtcc acggacattcctcgcaccatccgcggccaatcgagatatatcgcggtaaaccgatc ctgtacggatgcggtgacgtcgttgacgactacgaaggcatcggcgggcacgagtc gttccgcagtgaactgcgactgctgtatctgaccgtcaccgatcccgccagcggga acctgatctcgctgcagatgcttccactgcgagtgtcgcggatgcgcctacagcgt gcctcccagaccgacaccgaatggctccgcaacaccattgagcgcatcagccgccg gttcgggattcgagtcgtgactcgacccgacaacctgctggaggtcgttcccgctg ccaacctaacgagcaaggag
Rv1264	**52**	gtgacagaccacgtgcgcgaggcggacgacgcgaacatcgacgatctgttgggcga cctgggcggtaccgcgcgcgccgagcgtgcgaagcttgtcgagtggttgctcgagc agggcatcacccccgacgagattcgggcgaccaacccgccgttgctgctggccacc cgccacctcgtcggcgacgacggcacctacgtatccgcaagggagattagcgagaa ctatggcgttgacctcgagctgctgcagcgggtgcagcgcgctgtcggtctggcca gagtggatgatcctgacgcggtggtgcacatgcgtgccgacggtgaggcggccgca cgcgcacagcggttcgttgagctggggctgaatcccgaccaagtcgtgctggtcgt gcgtgtgctcgccgagggcttgtcacacgccgccgaggccatgcgctacaccgcgc tggaggccattatgcggccgggggctaccgagttggacatcgcgaaggggtcgcag gcgctggtgagccagatcgtgccgctgctggggccgatgatccaggacatgctgtt catgcagctgcggcacatgatggagacggaggccgtcaacgccggagagcgtgcgg ccggcaagccgctaccgggagcgcgacaggtcaccgttgccttcgccgacctggtc ggtttcacccagctaggcgaagtggtgtcggccgaagagctagggcacctcgccgg gcggctggccggcctcgcgcgtgacctgaccgctccgccggtgtggttcattaaga cgatcggcgacgcggtcatgttggtctgtcctgatccggcgccattgctggacacc gtgctgaagctggtcgaggtcgtcgacaccgacaacaactttccccggctgcgagc cggcgtcgcctccgggatggcggttagccgggccggcgactggttcggcagcccgg tcaacgtggcaagccgggtgaccggggtggcgcgcccgggtgccgtgctggtcgcg gattcggtgcgggaggcccttggtgatgccccgaagccgacggatttcagtggtc cttcgccggccccgtcgcctcaggggaatccggggtgacgtcaggctttttcgag tccggcgaggggccactcgcaccggctccggcggcgcggcccaagacgacgatttg gccggctcgtcaccg

Table 2. (continued) DNA sequences of selected low oxygen induced antigens		
Rv no.	SEQ ID NO:	Sequence
Rv1592c	**53**	atggtagagcccggcaatttggcaggcgcgaccggcgccgaatggatcggccggcc accgcacgaggaattgcagcgcaaagtgcgcccgctgctgccatccgacgatccgt tctacttcccacctgccggctaccagcatgccgtgcccggaacggtgttgcgctcg cgcgatgtcgaactggcgtttatgggcttgattccgcagcccgtcaccgctaccca gctgctgtaccggaccacgaacatgtacggcaaccccgaggcgacggtgaccacgg tgatcgtcccagcggagcttgccccgggtcagacctgccccttgctgtcgtaccag tgtgcgatcgatgccatgtcgtcgcgctgttttccgtcatatgccctgcgacgacg ggccaaggccctggggtcactgacccaaatggagctgttgatgatcagcgccgcac ttgccgaaggatgggcggtatcagtacccgaccatgaagggccgaaagggctgtgg gggtcgccgtatgaacccggttaccgagtcctcgacggaatccgggctgccttgaa ttccgagcgtgtcgggttgtccccggcaacgccgatcgggctgtggggctactccg gcggcgggctggccagcgcgtgggccgccgaagcatgcggcgagtacgcaccggac ctagacatcgtcggcgccgtgctgggatcacccgtcggtgaccttggtcacacgtt ccgccggctcaatggcactcttcttgccggtctgcccgcgttggtggtggccgcgc tgcaacacagctaccccggcctggcccgggtgatcaaggagcacgccaacgacgaa ggacgtcagctgctggagcaactgacggagatgacaacggtagacgcagtgatccg gatggccggcagggacatgggtgacttcctcgacgaacccccttgaggacattctgt cgacgccggaaatttcccatgtcttcggcgacaccaagctgggtagcgcggtgccc accccgccggtattgatcgtgcaggccgtgcatgactacctcatcgacgtctctga catcgacgcgctcgctgacagctatacagccggcggcgccaacgtcacctaccacc gcgacctgttcagcgaacatgtgtccctgcacccgctgtcggccccaatgacgctt cgctggctcaccgaccggttcgccggcaagccactgaccgaccaccgcgtccggac cacgtggccgaccatcttcaacccgatgacctacgccggcatggcgagactggccg tgatcgcggccaaggtgatcaccggcaggaagttgagccgccgtccgctc
Rv1733c	**54**	atgatcgccacaacccgcgatcgtgaaggagccaccatgatcacgtttaggctgcg cttgccgtgccggacgatactgcgggtgttcagccgcaatccgctggtgcgtggga cggatcgactcgaggcggtcgtcatgctgctggccgtcacggtctcgctgctgact atcccgttcgccgccgcggccggcaccgcagtccaggattcccgcagccacgtcta tgcccaccaggcccagacccgccatcccgcaaccgcgaccgtgatcgatcacgagg gggtgatcgacagcaacacgaccgccacgtcagcgccgccgcgcacgaagatcacc gtgcctgcccgatgggtcgtgaacggaatagaacgcagcggtgaggtcaacgcgaa gccgggaaccaaatccggtgaccgcgtcggcatttgggtcgacagtgccggtcagc tggtcgatgaaccagctccgccggcccgtgccattgcggatgcggccctggccgcc ttgggactctggttgagcgtcgccgcggttgcgggcgccctgctggcgctcactcg ggcgattctgatccgcgttcgcaacgccagttggcaacacgacatcgacagcctgt tctgcacgcagcgg
Rv1734c	**55**	atgaccaacgtcggtgaccaggggttgacgcggtcttcggggtgatctacccacc tcaggtcgcgctggtcagtttcggcaagccggcacaacgagtttgcgccgtcgacg gcgcgatccacgtcatgacgaccgtgctggctacgctgcccgctgaccacggctgc agcgatgaccatcgcggcgcgctgttcttcctgtcgatcaacgagctgacgcggtg cgccgcagtaacagga

Table 2. (continued) DNA sequences of selected low oxygen induced antigens		
Rv no.	**SEQ ID NO:**	**Sequence**
Rv1736c	**56**	gtgacggtgacaccacggaccggcagccgcatcgaggagctgcttgcacgcagcgg ccggttcttcatcccgggtgagatctcggcggatctgcgtaccgtgacccgccgcg gcggccgcgacggcgacgtgttctatcgagaccggtggagccacgacaaggtggtc cgctccacacacggggtgaattgcaccgggtcgtgttcttggaagatctacgtcaa agacgacatcatcacctgggagacgcaggagaccgactatccgtcggtgggcccgg accggcccgagtatgagccccgcggctgcccgcgcggcgcggcgttttcctggtac acgtattcgccgacgcgggtgcgccatccgtacgcccgcggcgtgcttgtcgagat gtatcgggaggcgaaggcacgtttgggtgatccggtggcggcctgggccgacatcc aggccgacccgcggcggcgccgccgctaccagcgcgcccgcggcaagggcgggctg gtccgggtcagctgggccgaggccaccgagatgatcgccgccgcccacgtgcacac catctccacatacggcccggaccgggttgccggcttctcccccatcccggcgatgt ccatggtgagccacgccgcggggtcgcggttcgtggagctaatcggcggggtgatg acgtcgttctacgactggtacgccgacctgccggtggcctccccgcaggtgttcgg cgaccagaccgacgtgccggagtccggagattggtgggacgtggtgtggcaatgcg cctcggtgctgctgacctacccgaactcacggcaactcggcaccgcagaggaattg ctggcccacatcgacggtccggccgcggatctgttggggcgcacggtctctgagct gcgccgtgccgatccgctgaccgcggcgacccgctacgtcgacaccttcgacctgc gaggccgcgccaccctgtacctgacctactggaccgccggcgacacccgcaaccgc ggccgggagatgctggccttcgcccagacctaccgcagcaccgacgtcgcaccacc gcgcggcgagaccccggacttcctgccggtggtgctcgaattcgccgcgaccgtcg accccgaggcggggcgacggttgctgagcgggtaccgggtgcccatcgccgcgctg tgcaatgccctgaccgaggccgcattgccatacgcacacacggtggccgcggtatg ccggacgggtgacatgatgggcgaactcttctggaccgtcgtgccgtatgtgacga tgacgatcgtcgcggtcggctcctggtggcgctaccgctatgacaaattcggctgg accacccgctcgtcccagctgtacgagtcgcggctgctgcggatcgccagcccgat gtttcatttcggcatcctggtggtcatcgtcggccacggtatcgggctcgtgatcc cgcagtcgtggactcaggccgccggtttgagcgagggcgcatatcacgtgcaggcc gtcgtgctggggtcgatcgccggcatcaccaccttggccggcgttaccctgctgat ctaccggcggcgcacccgcgggccggtgttcatggctaccaccgtcaacgacaagg tgatgtacctcgtgctggtggcggcgatcgtcgcgggactgggtgcgacggcgttg ggctccggcgttgtcggcgaggcgtacaactaccgcgagacggtgtcggtgtggtt ccgctcggtgtgggtactgcaaccgcgcggggacctgatggccgaggctccgctgt attaccagatccatgtgctgatcgggttggcgttgttcgcgttgtggccgttcacc cggctggtacacgcgttcagcgccccgatcggctatctgttccgcccgtacatcat ctaccgcagccgcgaggagctggtgctaacgcggccgcggcggcgcgggtgg

Table 2. (continued) DNA sequences of selected low oxygen induced antigens		
Rv no.	**SEQ ID NO:**	**Sequence**
Rv1737c	**57**	atgagagggcaagcggccaatctcgtgctggccacctggatctcggtggtcaactt ctgggcgtggaacctgatcggcccgctgtcgaccagctacgcgcgtgacatgtcac tgtccagcgccgaggcgtcgctgctcgtcgccaccccgatcctggtgggtgccctt ggccgcatcgtcaccgggccgctcaccgaccgcttcggcgggcgcgccatgctcat cgcggtgacgctggcgtcgatcctcccggtgctcgcggtcggggtcgcggcaacca tgggctcctacgcgttgctggtgtttttcgggctcttcctgggcgttgccggcacg atcttcgccgtcggcatcccgttcgccaacaactggtaccagccggcgcggcgcgg tttctccaccggcgtgttcggtatgggcatggtcggcaccgcgctctcggcgttct tcaccccgcggtttgtacggtggttcggcctgttcaccacccacgccatcgtcgcg gccgcgctcgcgtcgaccgccgtggtggccatggtcgtgcttcgtgatgcacccta ctttcggcccaacgccgacccggtgctgcccaggctcaaggccgcggcacggttgc cggtgacctgggagatgtcgtttctgtacgcgatcgtgttcggcgggttcgtggcg ttcagcaactacctgcccacctacatcaccacgatctacgggttctccacggtcga cgcgggcgctcgcaccgccgggttcgccctggcggcggtgctggcccggccggtgg gcgggtggctctccgaccggatcgcaccgaggcacgtggtgctggcctcgctcgcc gggaccgcgctgctggcgttcgccgcggcgttgcagccgccgccggaggtgtggtc ggcggccaccttcatcaccctggcggtctgcctcggcgtgggcaccggcggcgtgt tcgcgtgggtggcccgccgcgccccggccgcatcggtcggctcggtcaccggaatc gtcgccgcggcaggcggattgggcggttacttcccgccgctggtgatgggcgcgac ctacgacccggtcgacaacgactacacggtcgggttgctgctgctggtggcgaccg cgctggtcgcgtgtacctacaccgcgctgcacgcgcgggagccggtgagtgaggag gcgtccagg
Rv1738	**58**	atgtgcggcgaccagtcggatcacgtgctgcagcactggaccgtcgacatatcgat cgacgaacacgaaggattgactcgggcgaaggcacggctgcgttggcgggaaaagg aattggtgggtgttggcctggcaaggctcaatccggccgaccgcaacgtccccgag atcggcgatgaactctcggtcgcccgagccttgtccgacttggggaagcgaatgtt gaaggtgtcgacccacgacatcgaagctgttacccatcagccggcgcgattgttg- tat

Table 2. (continued) DNA sequences of selected low oxygen induced antigens

Rv no.	SEQ ID NO:	Sequence
Rv1739c	**59**	atgattcccacgatgacatcggccggctgggcaccaggggtggtgcagttccgcga ataccaacggcgttggctgcgcggcgatgtcctcgccggcctgaccgtggccgcct atctgatcccgcaagcgatggcgtatgcgaccgtggcgggcctaccgccggcagcc gggctgtgggcgtcgatcgcgccgcttgccatttacgcactgctcggatcgtcccg gcagctttcaatcggcccggaatccgccaccgccttgatgacggcggccgtgctcg ctccgatggccgccggggatcttcgacgctatgccgttctggcggcaaccctcgga ttgctagtcggccttatctgcctactcgctggcacggcgcgactaggtttcctcgc cagcctgcgatcgcggccggtgctcgtcggatacatggccggcatcgcgcttgtca tgatctccagccaactcggcactatcaccggcacctcggtcgaaggcaacgaattc ttcagcgaagtacactctttcgcgactagcgtcacgcgagttcactggccgacttt tgtgttagccatgtctgtcctagcgctgctaactatgctcacgcggtgggcgccgc gcgcccccggaccgatcatcgcggttcttgcggccacgatgctagtggccgttatg tccttggatgccaaaggtattgcgattgtgggtcggataccttccggtctgccgac gccgggtgtgccgcccgtttcggtggaagacttgcgggcactgatcattccggctg ccgggatcgcgattgttaccttcaccgacggtgtgttgaccgcacgcgccttcgcc gctcgtcgaggtcaggaagtcaatgccaacgccgagctgcgcgcggtcggggcctg caacatcgccgccgggctgacacacggttttccggtgagttccagcagcagccgta ccgccctcgccgacgtcgtcggtggccgcacccagctgtactcgctgatcgcgttg gggcttgttgtcatcgtgatggttttcgcgagtgggctgctggccatgtttccgat cgccgctctgggcgctttggtggtatatgccgcgctacgcttgatcgacttgtcag aattccggcgactggcgcggtttcggcgcagcgaactcatgctggcactagccacc acagcagccgtgttaggcctaggagtgttctatggagtcctcgccgcggttgccct gtccatcctcgaactgcttcgtcgggtcgcacatccgcatgacagcgttctcgggt tcgtgccgggcattgccggcatgcacgacatcgatgactatccgcaggccaagcgc gtgcccgggctggtggtgtatcgctatgacgcgccgttgtgcttcgccaatgccga agacttccgcaggcgagcactgaccgtggtcgatcaggatccggggcaagtcgagt ggttcgtactcaacgccgaatccaatgtggaggtcgacctgactgcgctggatgcg ctcgaccaactccgcaccgagctgctgcgtcggggaatagtgttcgccatggcccg ggtcaaacaagacttgcgtgaatcactcagggccgccagtcttctcgataagattg gcgaagaccatatctttatgacattgcctaccgcagtgcaggcgttccgtcggcgc
Rv1813c	**60**	atgatcacaaacctccgacgccgaaccgcgatggcagccgccggcctaggggctgc tctcgggctgggcatcctgctggttccgacggtggacgcccatctcgccaacggtt cgatgtcggaagtcatgatgtcggaaattgccgggttgcctatccctccgattatc cattacggggcgattgcctatgcccccagcggcgcgtcgggcaaagcgtggcacca gcgcacaccggcgcgagcagagcaagtcgcactagaaaagtgcggtgacaagactt gcaaagtggttagtcgcttcaccaggtgcggcgcggtcgcctacaacggctcgaaa taccaaggcggaaccggactcacgcgccgcgcggcagaagacgacgccgtgaaccg actcgaaggcgggcggatcgtcaactgggcgtgcaac

Table 2. (continued) DNA sequences of selected low oxygen induced antigens		
Rv no.	**SEQ ID NO:**	**Sequence**
Rv1997	**61**	ttgtcggcgtcagtgtctgccacgacggctcatcatggcttgccagcacatgaagt ggtgctgctgctggagagcgatccatatcacgggctgtccgacggcgaggccgccc aacgactagaacgcttcgggcccaacaccttggcggtggtaacgcgcgctagcttg ctggcccgcatcctgcggcagtttcatcacccgctgatctacgttctgctcgttgc cgggacgatcaccgccggtcttaaggaattcgttgacgccgcagtgatcttcggtg tggtggtgatcaatgcgatcgtgggtttcattcaagaatccaaggcagaggccgca ctgcagggcctgcgctccatggtgcacacccacgccaaggtggtgcgcgagggtca cgagcacacaatgccatccgaagagctggttcccggtgaccttgtgctgttagcgg ccggtgacaaggttcccgccgatttgcggctggtgcgacagaccggattgagcgtg aacgagtcagcacttaccggcgagtcgacgccggttcacaaggacgaggtggcgtt gccggagggcacaccggtcgctgatcgtcgcaatatcgcgtattccggcacattgg taaccgcgggccatggcgccgggatcgtcgtcgcgaccggcgccgaaaccgaactc ggtgagattcatcggctcgttggggccgccgaggttgtcgccacaccgctgaccgc gaagctggcgtggttcagcaagtttctgaccatcgccatcctgggtctggcagcgc tcacgttcggcgtgggtttgctgcgccggcaagatgccgtcgaaacgttcaccgct gcgatcgcgctggcggtcggggcaattcccgaaggtctgcccaccgccgtgaccat caccttggccatcggcatggcccggatggccaagcgccgcgcggtcattcgacgtc tacccgcggtggaaacgctgggcagcaccacggtcatctgcgccgacaagaccgga acgctgaccgagaatcagatgacggtccagtcgatctggacaccccacggtgagat ccgggcgaccggaacgggctatgcacccgacgtcctcctgtgcgacaccgacgacg cgccggttccggtgaatgccaatgcggcccttcgctggtcgctgctggccggtgcc tgcagcaacgacgccgcactggttcgcgacggcacacgctggcagatcgtcggcga tcccaccgagggcgcgatgctcgtcgtggccgccaaggccggcttcaacccggagc ggctggcgacaactctgccgcaagtggcagccataccgttcagttccgagcggcaa tacatggccaccctgcatcgcgacgggacggatcatgtggtgctggccaagggtgc tgtggagcgcatgctcgacctgtgcggcaccgagatgggcgccgacggcgcattgc ggccgctggaccgcgccaccgtgttgcgtgccaccgaaatgttgacttcccggggg ttgcgggtgctggcaaccgggatgggtgccggcgccggcactcccgacgacttcga cgaaaacgtgataccaggttcgctggcgctgaccggcctgcaagcgatgagcgatc caccacgagcggccgcggcatcggcggtggcggcctgccacagtgccggcattgcg gtaaaaatgattaccggtgaccacgcgggcaccgccacggcgatcgcaaccgaggt ggggttgctcgacaacactgaaccggcggcaggctcggtcctgacgggtgccgagc tggccgcgctgagcgcagaccagtacccggaggccgtggatacagccagcgtgttt gccagggtctctcccgagcagaagctgcggttggtgcaagcattgcaggccagggg gcacgtcgtcgcgatgaccggcgacggcgtcaacgacgccccggccttgcgtcagg ccaacattggcgtcgcgatgggccgcggtggcaccgaggtcgccaaggatgccgcc gacatggtgttgaccgacgacgacttcgccaccatcgaagccgcggtcgaggaagg ccgcggcgtattcgacaatctgaccaagttcatcacctggacgctgcccaccaacc tcggtgagggcctagtgatcttggccgccatcgctgttggcgtcgccttgccgatt ctgcccacccaaattctgtggatcaacatgaccacagcgatcgcgctcggactcat gctcgcgttcgagcccaaggaggccggaatcatgacccggccaccgcgcgaccccg accaaccgctgctgaccggctggcttgtcaggcggactcttctggtttccaccttg ctcgtcgccagcgcgtggtggctgtttgcatgggagctcgacaatggcgcgggcct gcatgaggcgcgcacggcggcgctgaacctgttcgtcgtcgtcgaggcgttctatc tgttcagctgccggtcgctgacccgatcggcctggcggctcggcatgttcgccaac cgctggatcatcctcggcgtcagtgcgcaggccatcgcgcaattcgcgatcacata tctacccgcgatgaatatggtgttcgacaccgcgccaatcgatatcggggtgtggg tgcgcatattcgctgtcgcgaccgcaatcacgattgtggtggccaccgacacgctg ctgccgagaatacgggcgcaaccgcca

Table 2. (continued) DNA sequences of selected low oxygen induced antigens		
Rv no.	**SEQ ID NO:**	**Sequence**
Rv1998c	**62**	atgagtttccacgatcttcatcaccaaggtgttccgttcgtgttgcccaacgcctg ggatgtgccgtcggccctggcctacctcgcggagggcttcacggctatcggcacaa ccagtttcggggtctcgtccagcggcgggcacccggacgggcaccgcgccactcgc ggcgccaacatcgcactggcggccgccctggcaccgctgcaatgctacgtcagcgt cgacatcgaggacggatacagcgacgaacccgacgccattgctgactacgtcgcac aactgtcgacagccggaatcaatatcgaggacagtagcgccgaaaagctcatcgac cccgccctggcagccgctaaaatcgttgcgatcaaacaacgtaaccccgaggtgtt cgtcaacgcccgcgtcgacacctattggttgcgccagcacgccgataccaccagca cgatccagcgcgcacttcgctacgtcgatgccggcgccgacggcgtctttgtccca ctggccaacgatcccgacgaacttgctgagctcactcgcaacattccgtgcccggt taacacgttgcccgtgcccggcttgacgatcgccgaccttggtgagctcggggtgg cccgggtgtcaaccggttcagtgccctacagcgcggggttgtatgcagcggcccac gcggctcgggccgtgagcgacggagagcagctgccacggtccgtaccgtacgccga actgcaggcacgcttggttgactacgagaaccgcacgagtacaacg
Rv2003c	**63**	gtggtcaagcgctctcgggcaacccgactttcgccgagcatctggtccggatggga atcacctcagtgtcggtccattcgggcgcgattgctgctaccccggggtcggtcgc ggccgccgaacgccgattgttgctggaatcagctcgcggtgacgcctgacacccgg atgccggcatcgtcggccgccgggcgcgacgcggcggcctacgacgcctggtatga ctcacccaccgggcggccgatcctggcgaccgaggtcgccgcgttgcggccgctca tcgaggtctttgcccagccacgcttggaaatcggtgtcggtacaggacgtttcgcc gacctgctcggcgtgcggttcggactcgatccatcccgtgatgcgctgatgttcgc acgccggcgcggcgtcctggtcgccaatgccgtcggcgaggcggtccctttcgtca gccggcacttcggggcggtcctcatggcattcacgctctgtttcgtcaccgacccg gccgccatattccgggaaacgcggcgtctgctcgccgacggcggcggccttgttat cgggttcttgcctcgcgggacaccgtgggccgacctgtacgctctgcgcgcggccc gcggacagccaggctaccgcgacgcccgcttctacaccgcggccgaactcgaacaa ctgctcgcagactcgggattccgggtcatcgcccgccgctgcacgctgcaccaacc gccgggactcgcccggtacgacatcgaagccgcccatgacggtatccaagccggcg ccggcttcgttgctatctcggcggtcgaccaagcgcacgagcctaaggatgatcac ccactcgagtcggaa
Rv2005c	**64**	atgtctaaaccccgcaagcagcacggagttgtcgtcggggtagatggttcgctcga atcggatgccgccgcctgttggggtgccaccgatgcggcgatgaggaacattccgc tgaccgtggtccacgtggtgaacgccgatgtagcgacgtggccgccgatgccgtat ccggagacctgggggttggcaggaggacgagggtcgccagatcgtcgccaacgc cgtcaagctcgccaaagaggcggttggagcggatcgaaagctcagcgtaaagagcg agctcgtattttccacgccggtacctaccatggttgaaatctccaacgaggcagag atggtggtgttgggcagctcgggccggggagcgctggcccgaggcttgctcggttc ggtcagctcgagcctggtgcgacgcgccgggtgcccggtcgcggtcatccacagcg atgatgcggtgatccctgatccgcagcacgctcccgtgctggtgggaatcgacggt tcgccggtttcggagcttgcgacggcggtggcatttgacgaggcgtcgcgccgcgg cgtcgaactgatcgccgtgcacgcgtggagtgacgtcgaagtggtggaacttccgg gtttggacttctcggctgtacagcaggaagcggagcttagtctcgccgaacgcttg gcaggttggcaagaacgctatcccgatgtgccggtgagccgggttgtcgtttgcga tcgcccggcgcggaagctggtgcaaaagtcggcgtccgcccagcttgtcgtcgttg gcagtcatggccgaggtggcttgaccggcatgcttctggggtcggtcagtaacgcg gtcttacacgccgcgcgggtgccagtgatcgtggcacggcagtcg

Table 2. (continued) DNA sequences of selected low oxygen induced antigens		
Rv no.	**SEQ ID NO:**	**Sequence**
Rv2007c	**65**	gtgacctatgtgatcggtagtgagtgcgtggatgtgatggacaagtcctgtgtgca ggagtgtccggtcgactgtatctatgagggcgcccgaatgctctacatcaaccccg acgagtgcgtggattgtggtgcgtgcaaaccggcctgccgcgtcgaggcgatctac tgggaaggcgatctacccgacgatcaacaccagcatctgggggacaacgccgcctt tttccaccaagtcctgccgggccgagtggctccgctgggttcgccgggtggtgccg cagcggtgggcccgatcggagtcgacacgcctctggtcgcggctatcccggtg- gagtgccct
Rv2028c	**66**	atgaaccaatcacacaaacccccatcgatcgtcgtcggtattgatggctcgaagcc ggccgtgcaagccgcactgtgggcggtcgacgaggcagccagccgtgacatcccgc tgcgtctgctgtacgcgatcgaacccgacgatcccgggtacgccgcacacggcgcg gcggctcgcaaactcgccgccgccgagaacgcggtgcgctacgcgttcacagcggt cgaggcggcggaccggccggtcaaggtcgaggtggagatcacccaggagcggccgg tcacctcgttgatccgcgcttcggcggctgctgccctggtgtgcgttggcgctatc ggcgtgcaccacttccgaccggagcgggtgggatctaccgcagcggccctggcgtt atcggcgcagtgcccagtggcgatcgtgcgaccccaccgggtccccatcggacgcg acgccgcatggatcgtcgtcgaggcggacgggtcgtccgatatcggtgttttgctg ggggcggtgatggccgaagcacggctgcgcgactcgccggttcgggtggtcacctg ccggcaatccggagtgggcgataccggggacgacgtccgtgccagcctggaccgct ggcttgcccgttggcaaccacggtatcccgatgtgcgggtgcaatcggcggcagtg cacggcgagctgctggattatctggctgggctgggtcgatcggtacacatggtggt gctcagcgcgagcgaccaggagcatgtggagcaacttgtgggagcgccgggcaacg ccgtgttgcaggaggccggctgcaccctgctggtcgtcggtcagcagtatctg
Rv2029c	**67**	atgacggagccagcggcgtgggacgaaggcaagccgcgaatcatcactttgaccat gaaccccgccttggacatcacgacgagcgtcgacgtggtgcgcccgaccgagaaaa tgcgttgtggcgcacctcgctacgatcccggcggcggcggtatcaatgtcgcccgc attgtgcatgtcctcggcggttgctcgacagcactgttcccggccggcgggtcgac cgggagcctgctgatggcgctgctcggtgatgcgggagtgccatttcgcgtcattc cgatcgcggcctcgacgcgggagagcttcacggtcaacgagtccaggaccgccaag cagtatcgtttcgtgcttccggggccgtcgctgaccgtcgcggagcaggagcaatg cctcgacgaactgcgcggtgcggcggcttcggccgcctttgtggtggccagtggca gcctgccgccaggtgtggctgccgactactatcagcgggttgccgacatctgccgc cgatcgagcactccgctgatcctggatacatctggtggcgggttgcagcacatttc gtccggggtgtttcttctcaaggcgagcgtgcgggaactgcgcgagtgcgtcggat ccgaactgctgaccgagcccgaacaactggccgccgcacacgaactcattgaccgt gggcgcgccgaggtcgtggtggtctcgcttggatctcagggcgcgctattggccac acgacatgcgagccatcgattttcgtcgattccgatgaccgcggttagcggtgtcg gcgccggcgacgcgatggtggccgcgattaccgtgggcctcagccgtggctggtcg ctcatcaagtccgttcgcttgggaaacgcggcaggtgcagccatgctgctgacgcc aggcaccgcggcctgcaatcgcgacgatgtggagaggttcttcgagctggcggccg aacccaccgaagtcgggcaggatcaatacgtttggcacccgatcgttaacccggaa gcctcgcca

Table 2. (continued) DNA sequences of selected low oxygen induced antigens		
Rv no.	**SEQ ID NO:**	**Sequence**
Rv2030c	**68**	gtgctgatgaccgcagcggctgatgtcacccggcgctcgccgcggcgcgtgttccg tgaccgccgcgaggccggccgggtgctggcggaattactcgccgcctatcgggacc agccggacgtgattgtgctcggcttggcccggggtggcctcccggtcgcatgggag gttgccgcggcactgcatgccccgctagacgccttcgtcgtgcgcaaacttggtgc cccggggcatgacgagttcgccgttggtgcactggccagcggcggccgcgtcgtgg tcaatgacgacgtcgtgcggggcctgcggatcacaccgcagcaactgcgcgacatc gccgaacgtgagggtcgggaactgcttcggcgcgagtccgcctaccgcggcgagcg cccgcccaccgatatcaccggcaagacggtcattgtcgtcgatgacggtttggcca ccggcgcaagcatgttcgcggcggtacaggcattgcgcgatgcgcaaccagcgcag atcgtgattgccgtgccggcggcgccggagtccacgtgccgggagttcgccggcct cgtcgacgacgttgtgtgcgcgaccatgccgaccccgttcctggccgtcggtgagt cgttttgggacttccggcaggtcaccgacgaggaggtccgccggctcctggccacc ccgaccgctgggccgtcgctgcgccggcccgcggcgtcaacggcggccgatgttct gcgcagagtcgcgatcgacgcccccgggggtgttccgacgcacgaggtgttggcgg agctggtcggcgatgcacgaatcgtgttgatcggcgaaagctcgcacggcacacac gagttctaccaggcccgggccgccatgacacagtggctgatcgaggagaagggctt tggtgcggtagccgccgaggcggactggcccgacgcctaccgggtcaatcggtacg ttcgcggcctcggcgaggacaccaacgctgacgaggcgcttagcggattcgagcgg tttcccgcctggatgtggcgcaacaccgtggtccgagattttgtggaatggctgcg cacacgcaaccagcgctacgagtcgggcgcgctgcggcaagccggcttctacggtc tggatctttacagcctgcatcggtcgatccaagaggtgatcagctatctcgacaag gtcgacccgcgtgcggcggcacgggcgcgggcccggtatgcgtgcttcgaccatgc ctgcgccgatgacggtcaggcgtacggattcgcggccgcattcggcgccggtccgt cgtgcgaacgtgaagccgtcgagcaactggtcgacgttcagcgcaatgccctggcg tatgcgcgccaagacgggctgcttgccgaggacgaactgttctacgcccagcaaaa cgcgcagacggtgcgcgacgcagaggtgtattaccgggccatgttcagtggacgcg ttacctcgtggaacctgcgcgaccagcacatggcgcagacccttggcagtttgctg acgcatttggaccgacacctcgatgcgccgccggcgcgaatagtggtgtgggctca taactcccacgtgggtgacgcacgcgctaccgaggtgtgggccgacgggcagctca ccctcggccagatagtccgtgagcgatacggtgacgagtcgcgcagcatcggattc agcacgtacacgggcaccgtcaccgcggccagcgaatggggtggtatcgcccaacg caaagcggttcggccggcactgcacggcagtgtcgaggagctcttccaccagactg cagacagtttcctggtgtcagcgcggctaagccgcgacgccgaagccccgctggac gttgtccggttgggacgtgccatcggcgtcgtttatctaccggcaacggaacggca aagtcactacttgcacgtgcggcccgccgaccagttcgacgccatgatccacatcg atcagacccgtgccctggaacctctcgaggtgacgagccggtggatcgccggcgag aacccggaaacctacccgaccggtctg
Rv2031c	**69**	Atggccaccacccttcccgttcagcgccacccgcggtccctcttccccgagttttc tgagctgttcgcggccttcccgtcattcgccggactccggcccaccttcgacaccc ggttgatgcggctggaagacgagatgaaagaggggcgctacgaggtacgcgcggag cttcccggggtcgaccccgacaaggacgtcgacattatggtccgcgatggtcagct gaccatcaaggccgagcgcaccgagcagaaggacttcgacggtcgctcggaattcg cgtacggttccttcgttcgcacggtgtcgctgccggtaggtgctgacgaggacgac attaaggccacctacgacaagggcattcttactgtgtcggtggcggtttcggaagg gaagccaaccgaaaagcacattcagatccggtccaccaac

Table 2. (continued) DNA sequences of selected low oxygen induced antigens		
Rv no.	**SEQ ID NO:**	**Sequence**
Rv2032	**70**	atgccggacaccatggtgaccaccgatgtcatcaagagcgcggtgcagttggcctg ccgcgcaccgtcgctccacaacagccagccctggcgctggatagccgaggaccaca cggttgcgctgttcctcgacaaggatcgggtgctttacgcgaccgaccactccggc cgggaagcgctgctggggtgcggcgccgtactcgaccactttcgggtggcgatggc ggccgcgggtaccaccgccaatgtggaacggtttcccaaccccaacgatcctttgc atctggcgtcaattgacttcagcccggccgatttcgtcaccgagggccaccgtcta agggcggatgcgatcctactgcgccgtaccgaccggctgcctttcgccgagccgcc ggattgggacttggtggagtcgcagttgcgcacgaccgtcaccgccgacacggtgc gcatcgacgtcatcgccgacgatatgcgtcccgaactggcggcggcgtccaaactc accgaatcgctgcggctctacgattcgtcgtatcatgccgaactcttttggtggac aggggcttttgagacttctgagggcataccgcacagttcattggtatcggcggccg aaagtgaccgggtcaccttcggacgcgacttcccggtcgtcgccaacaccgatagg cgcccggagtttggccacgaccgctctaaggtcctggtgctctccacctacgacaa cgaacgcgccagcctactgcgctgcggcgagatgctttccgccgtattgcttgacg ccaccatggctgggcttgccacctgcacgctgacccacatcaccgaactgcacgcc agccgagacctggtcgcagcgctgattgggcagcccgcaactccgcaagccttggt tcgcgtcggtctggccccggagatggaagagccgccaccggcaacgcctcggcgac caatcgatgaagtgtttcacgttcgggctaaggatcaccgg
Rv2428	**71**	atgccactgctaaccattggcgatcaattccccgcctaccagctcaccgctctcat cggcggtgacctgtccaaggtcgacgccaagcagcccggcgactacttcaccacta tcaccagtgacgaacacccaggcaagtggcgggtggtgttcttttggccgaaagac ttcacgttcgtgtgccctaccgagatcgcggcgttcagcaagctcaatgacgagtt cgaggaccgcgacgcccagatcctgggggtttcgattgacagcgaattcgcgcatt tccagtggcgtgcacagcacaacgacctcaaaacgttacccttcccgatgctctcc gacatcaagcgcgaactcagccaagccgcaggtgtcctcaacgccgacggtgtggc cgaccgcgtgacctttatcgtcgaccccaacaacgagatccagttcgtctcggcca ccgccggttcggtgggacgcaacgtcgatgaggtactgcgagtgctcgacgccctc cagtccgacgagctgtgcgcatgcaactggcgcaagggcgacccgacgctagacgc tggcgaactcctcaaggcttcggcc
Rv2624c	**72**	atgtctgggagaggagagccgacgatgaaaacaatcattgttggtatcgatggttc gcacgcggcgattacggccgcattgtgggggttgacgaggccatcagccgagcgg tgccgctgcgactggtctcagtgatcaagccgacacatccgtccccggacgactac gaccgcgaccttgcgcatgctgaaagatcgcttcgggaagcgcagtccgctgttga ggccgcgggcaagctcgtcaagatcgaaaccgacatcccccgcgggccagccggcc cggtgcttgtggaggcatcgcgcgacgccgagatgatctgcgtcggctccgtggga atcgggcgctacgccagctcgatcttgggttcgacggcaaccgagctggccgaaaa ggcgcattgcccggtcgccgtcatgcgctcaaaagtggaccagccagcgtctgaca tcaactggatcgtggtgcgcatgaccgacgcaccggataacgaggccgtgctggaa tacgctgcccgggaagcgaagttgcggcaagcgcccatactggcactcggcgggcg accggaggagctccgggagattccggacggcgaattcgaacgtcgcgtgcaggatt ggcaccaccgtcatcccgatgtgcgcgtctacccgatcaccactcacacgggtatt gcccggttcctggccgaccacgacgagcgcgtacagctggcagtgatcggcggtgg tgaggccggtcagctagcgcggctggtcgggccatccggacatccggtgttccgtc acgccgagtgttcggtgcttgtcgttcgccgc

Table 2. (continued) DNA sequences of selected low oxygen induced antigens		
Rv no.	**SEQ ID NO:**	**Sequence**
Rv2625c	**73**	atgcgtgatgcgatcccgcttgggcggatcgccgggtttgtggtgaacgtccactg gagcgtgttggtgatcctgtggttgttcacctggagtctggcgaccatgttgccgg gtaccgtcggaggctacccggccgtggtctattggcttctcggcgcaggtggcgcg gtcatgttgctggcgtcgctgttggctcatgagctcgcgcacgccgtcgtcgctcg tcgcgccggggtatccgttgagagcgtgacgttgtggctgttcggcggggtgaccg cgcttggcggcgaggcaaagacgcccaaagccgctttccggatcgcgttcgcgggt ccggctaccagcctggcgctgtcggcgacattcggtgcgttggccatcacgctcgc cggcgtgcggaccccggccatcgtgatcagcgttgcttggtggttggctactgtca acctgctgctggggctgttcaatctgctgcctggcgcgccgttggacggtgggcgg ttggtccgggcctatctgtggcgccgccacggcgatagtgtgcgcgccgggatcgg tgcggcgcgggccggacgggtggttgcgctggtcttgatcgcgttgggattggccg agtttgtggctggtggcctcgtcggtggggtctggttagccttcattggctggttt atcttcgctgccgctcgcgaggaggagacccggatttcgacccagcagctgtttgc cggggtgcgtgtggccgatgcgatgaccgcccaaccgcatacggctcccggatgga tcaatgtcgaggatttcatccagcgttacgtgcttggtgaacggcactcggcatat ccggttgccgatcgggacggatcgatcacgggcctggtggcattgcggcagctgcg cgatgttgcgcctagccggcgcagcactaccagcgtaggtgacattgcgctgccgc tgcacagcgtgccgaccgcccgaccacaagagccgctgaccgcgctcctagagcgg atggcaccgctcggcccgcgcagccgtgcgctggtcaccgaagggagcgcggtggt cggcatcgtcactcccagcgatgtcgcgcggctgattgacgtctaccggttggccc agccggaaccgacctttaccacgagtccccaagatgcggacaggttttccgatg- cgggg
Rv2627c	**74**	atggcaagttctgcgagcgacggcacccacgaacgctcggcttttcgcctgagtcc accggtcttgagcggcgccatgggaccgttcatgcacaccggtctgtacgtcgctc aatcgtggcgcgactatctgggtcaacagcccgataaactgccgatcgcacggccc actattgccttagcggcgcaagcctttcgagacgaaatcgtcctgctgggcctcaa ggcacgacgtccggtcagcaatcatcgagtgttcgagcgcatcagccaagaagtgg ccgctggactggagttctatgggaatcgcagatggctggagaagcctagcggattt tttgcccagcccccaccgctcaccgaggtcgcggtccgaaaggtcaaggaccgcag acgctccttttatcgcatcttcttcgacagtgggtttacgccgcatccgggtgaac cgggcagccaacggtggctctcatacactgcgaacaatcgcgagtacgccctgtta ctgcggcacccagagccgcgtccctggctggtttgtgtacacggcaccgagatggg cagggccccgttggatctcgcggtgttccgcgcctggaagctgcatgacgaactcg gcctgaacattgtcatgccggttcttccgatgcatggtccccgcgggcaaggtctg ccgaagggcgccgtttttcccggagaagatgttctcgacgatgtgcatgggacggc tcaagcggtgtgggatatccggcggctgttgtcctggatacgatcgcaggaggagg agtcgctgatcgggttgaacggtctctcgctgggcggctacatcgcgtcattggtc gccagcctcgaagaaggtctcgcctgcgcgattctcggtgtcccagtggctgatct gatcgagttgttgggccgccactgcggtcttcggcacaaagacccccgccgccaca ccgtcaagatggccgaaccgatcggccgaatgatctcgccgctctcacttacgcca ctggtgcccatgccgggccgctttatctacgcgggcattgccgaccgactcgtgca tccacgcgaacaggtgactcgcctctgggagcactggggcaaacccgaaatcgtgt ggtatccaggcggtcacactggcttcttccagtcgcggccggtacgacggtttgtc caggctgcgctggagcagtcgggcctgttggacgcgccacggacacagcgcgac- cgttccgcc

Table 2. (continued) DNA sequences of selected low oxygen induced antigens		
Rv no.	**SEQ ID NO:**	**Sequence**
Rv2628	**75**	Atgtccacgcaacgaccgaggcactccggtattcgggctgttggcccctacgcatg ggccggccgatgtggtcggataggcaggtgggggtgcaccaggaggcgatgatga atctagcgatatggcacccgcgcaaggtgcaatccgccaccatctatcaggtgacc gatcgctcgcacgacgggcgcacagcacgggtgcctggtgacgagatcactagcac cgtgtccggttggttgtcggagttgggcacccaaagcccgttggccgatgagcttg cgcgtgcggtgcggatcggcgactggcccgctgcgtacgcaatcggtgagcacctg tccgttgagattgccgttgcggtc
Rv2629	**76**	atgcgatcagaacgtctccggtggctggtagccgcagaaggtccgttcgcctcggt gtatttcgacgactcgcacgacactcttgatgccgtcgagcgccgggaagcgacgt ggcgcgatgtccggaagcatctcgaaagccgcgacgcgaagcaggagctcatcgac agcctcgaagaggcggtgcgggattctcgaccggccgtcggccagcgtggccgcgc gctgatcgcgaccggcgagcaagtactggtcaacgagcatctgatcggcccaccac cggctacggtgattcggctgtcggattatccgtacgtcgtgccattgatagacctt gagatgcggcgaccgacgtatgtatttgccgcggttgatcacaccggcgccgacgt caagctgtatcaggggccaccatcagttccacgaaaatcgatggggtcggctacc cggtgcacaagccggtcaccgccggctggaacggctacggcgacttccagcacacc accgaagaagccatccgaatgaactgccgcgcggtcgccgaccatctcacccgact ggtagacgctgccgaccccgaggtggtgttcgtgtccggcgaggtgcggtcacgca cagacctgctttccacattgccgcagcgggtggcggtccgggtgtcgcagctgcat gccggaccgcgcaaaagcgccttagacgaggaagagatctgggacctgacatccgc ggagttcacccggcggcggtacgccgaaatcaccaatgtcgcacaacaatttgagg cggagatcggacgcggatcggggctggcggcccaagggttggcggaggtgtgtgcg gctctgcgtgacggcgacgtcgacacgctgatcgtcggagagctaggcgaggccac cgtggtcaccggtaaagcgcgtactacggtcgcgcgggatgccgacatgttgtccg aactcggcgaaccggtagatcgcgtggcaagggccgatgaggcgttgccattcgcc gcgatcgcggtaggtgccgcattggtccgtgacgacaaccggatcgcgccactaga tggggtgggcgcattgctgcgttatgccgccaccaaccgactcggcagccata- gatcc
Rv2630	**77**	Atgctgcaccgcgacgatcacatcaatccgccgcggccccgcgggttggatgttcc ttgcgcccgcctacgagcgacaaatcccctgcgcgccttggcgcgttgcgttcagg cgggcaagccgggcaccagttcagggcatcggtccgtgccgcatacggcggacttg cgaatcgaagcctgggcaccgacccgtgacggctgtatccggcaggcggtgctggg taccgtcgagagcttcctcgacctggaatccgcgcacgcggtccatacccggctgc gccggctgaccgcggatcgcgacgacgatctactggtcgcggtgctcgaggaggtc atttatttgctggacaccgtcggtgaaacgcctgtcgatctcaggctgcgcgacgt tgacgggggtgtcgacgtcacattcgcaacgaccgatgcgagtacgctagttcagg tgggtgccgtgccgaaggcggtgtcactcaacgaacttcggttctcgcagggtcgc cacggctggcgatgtgcggtaacgctcgatgtg

Table 2. (continued) DNA sequences of selected low oxygen induced antigens		
Rv no.	**SEQ ID NO:**	**Rv no.**
Rv2659c	**78**	Gtgacgcaaaccggcaagcgtcagagacgcaaattcggtcgcatccgacagttcaa ctccggccgctggcaagccagctacaccggccccgacggccgcgtgtacatcgccc ccaaaaccttcaacgccaagatcgacgccgaagcatggctcaccgaccgccgccgc gaaatcgaccgacaactatggtccccggcatcgggtcaggaagaccgccccggagc cccattcggtgagtacgccgaaggatggctgaagcagcgtggaatcaaggaccgca cccgcgcccactatcgcaaactgctggacaaccacatcctggccaccttcgctgac accgacctacgcgacatcaccccggccgccgtgcgccgctggtacgccaccaccgc cgtgggcacaccgaccatgcgggcacactcctacagcttgctgcgcgcaatcatgc agaccgccttggccgacgacctgatcgactccaacccctgccgcatctcaggcgcg tccaccgcccgccgcgtccacaagatcaggcccgccaccctcgacgagctggaaac catcaccaaagccatgcccgacccctaccaggcgttcgtgctgatggcggcatggc tggccatgcgctacggcgagctgaccgaattacgccgcaaagacatcgacctgcac ggcgaggttgcgcgggtgcggcgggctgtcgttcgggtgggcgaaggcttcaaggt gacgacaccgaaaagcgatgcgggagtgcgcgacataagtatcccgccacatctga tacccgccatcgaagaccaccttcacaaacacgtcaaccccggccgggagtccctg ctgttcccatcggtcaacgaccccaaccgtcacctagcaccctcggcgctgtaccg catgttctacaaggcccgaaaagccgccggccgaccagacttacgggtgcacgacc ttcgacactccggcgccgtgttggctgcatccaccggcgccacactggccgaactg atgcagcggctaggacacagcacagccggcgccgcactccgctaccagcacgccgc caagggccgggaccgcgaaatcgccgcactgttaagcaaactggccgagaaccag- gagatg
Rv2780	**79**	Atgcgcgtcggtattccgaccgagaccaaaaacaacgaattccgggtggccatcac cccggccggcgtcgcggaactaacccgtcgtggccatgaggtgctcatccaggcag gtgccggagagggctcggctatcaccgacgcggatttcaaggcggcaggcgcgcaa ctggtcggcaccgccgaccaggtgtgggccgacgctgatttattgctcaaggtcaa agaaccgatagcggcggaatacggccgcctgcgacacgggcagatcttgttcacgt tcttgcatttggccgcgtcacgtgcttgcaccgatgcgttgttggattccggcacc acgtcaattgcctacgagaccgtccagaccgccgacggcgcactacccctgcttgc cccgatgagcgaagtcgccggtcgactcgccgcccaggttggcgcttaccacctga tgcgaacccaaggggccgcggtgtgctgatgggcggggtgcccggcgtcgaaccg gccgacgtcgtggtgatcggcgccggcaccgccggctacaacgcagcccgcatcgc caacggcatgggcgcgaccgttacggttctagacatcaacatcgacaaacttcggc aactcgacgccgagttctgcggccggatccacactcgctactcatcggcctacgag ctcgagggtgccgtcaaacgtgccgacctggtgattggggccgtcctggtgccagg cgccaaggcacccaaattagtctcgaattcacttgtcgcgcatatgaaaccaggtg cggtactggtggatatagccatcgaccagggcggctgtttcgaaggctcacgaccg accacctacgaccacccgacgttcgccgtgcacgacacgctgttttactgcgtggc gaacatgcccgcctcggtgccgaagacgtcgacctacgcgctgaccaacgcgacga tgccgtatgtgctcgagcttgccgaccatggctggcgggcggcgtgccggtcgaat ccggcactagccaaaggtctttcgacgcacgaaggggcgttactgtccgaacgggt ggccaccgacctgggggtgccgttcaccgagcccgccagcgtgctggcc

Table 2. (continued) DNA sequences of selected low oxygen induced antigens		
Rv no.	**SEQ ID NO:**	**Sequence**
Rv3126c	**80**	Atggtcatccggtttgatcaaatagggtcattggtcctctcaatgaaatcccttgc gtcactgtcgtttcagcggtgtctgcgcgagaattctagtttggtcgcggcgctgg accggctcgatgctgcggtcgatgagctgagcgctttgtcgtttgatgcgttgacc actccggagcgggatcgcgcccgtcgcgaccgggaccatcatccttggtcccgctc ccgctcgcagttgtcgccacgaatggcgcacggtgcagtgcaccaatgccagtggc cgaaggcggtttgggctgtcattgacaatcca
Rv3127	**81**	Gtgctcaagaacgcagtcttgctggcatgccgggcgccgtcggtgcacaacagcca gccctggcgttgggtggccgaaagcggctccgagcacactactgtgcacctgttcg tcaaccgccaccgaacggtgccggccaccgaccattccggccggcaagcgatcatc agttgcggtgccgtactcgatcaccttcgcatcgccatgacggccgcgcactggca ggcgaatatcactcgctttccccagccgaaccaacctgaccagttggccaccgtcg aattcagtcccatcgatcacgtcacggcgggacagcgaaaccgcgcccaggcgatt ctgcagcgccgaaccgatcggcttccgtttgacagcccgatgtactggcacctgtt tgagcccgcgctgcgcgacgccgtcgacaaagacgttgcgatgcttgatgtggtat ccgacgaccagcgaacacgactggtggtagcgtcacaactcagcgaagtcctgcgg cgggacgatccgtactatcacgccgaactcgaatggtggacttcaccgttcgtgct ggcccatggtgtgccgccggatacgctggcatcagacgccgaacgcttgcgggttg acctgggccgtgacttcccggtccggagctaccagaatcgccgtgccgagctagct gatgaccgatcgaaagtccttgtgctgtcgacccctagcgacacgcgagccgacgc actgaggtgtggcgaagtgctgtcgaccatcctactcgagtgcaccatggccggca tggctacctgcacgttgacccatctgatcgaatccagtgacagtcgtgacatcgtg cggggcctgacgaggcagcgaggcgagccgcaagccttgatccgggtagggatagc cccgccgttggcagcagttcccgcccccacaccacggcggccgctggacagcgtct tgcagattcgccagacgcccgagaaagggcgtaatgcctcagatagaaatgcccgt gaaacgggttggttcagcccgcct
Rv3128c	**82**	gtgtggtccgcctcgggtgggcagtgcgggaagtatcttgccgcctcgatggtgct gcagcttgatgggttggaacgtcacggtgtgttggagtttgggcgtgaccgctatg gccccgaggtgcgtgaggagctgttggcgatgagtgcggccagcatcgatcgttat ctgaagaccgcgaaggccaaagaccagatatcgggtgtgtcgacgacgaaaccctc accactgctgcgtaattcgatcaaggttcgcagggccggcgatgaggtcgaggcgg agccggggttcttcgagggcgacaccgtcgcccattgcggtccgacgctcaaaggc gagttcgcccacaccctgaacttgaccgacgtgcacatcggatgggtgttcacccg caccgtccgcaacaacgcccgtacccacatcctcgccgggctcaaagcttctgtca ccgagatcccgcatgggataacgggtttagatttcgacaacggcaccgtgtttctc aacaagccggtcatcagctgggccggcgacaacggtatctacttcacccgctttcg cccgtacaagaaaaaccactaggccaccatcgagtccaagaacaaccacctggtcc gcaagtacgcgttctactaccgctatgacaccgccgaggaacgcgccgtgctcaac cggatgtggaagctggtcaacgaccgcctcaactacctcaccccgaccatcaaacc gatcgggtatgccagcagcgccgacggccgccgccgacgcctctacgatgccccac agacgccgctggaccggccactggccgcaagggtgctctccgcggcccagcaggcc gacctgatcacctaccgagacagcctcaaccccgcccagatcggccgcaaaatcgc cgacctgcagaaccgactcctcatcttggccaaggagaaaaccgagcagctctacc tcgctaacatccccaccgccctacccgacatccacaaaggcatcctgatcaagg- cgggc
Rv3129	**83**	Gtggtgcaaggccgcaccgtgctgtttcgtaccgcggagggcgccaaattattttc agccgtcgcgaagtgcgcggtggctttcgaggcggacgaccacaacgttgccgagg gctggagcgtgatcgtcaaggttcgcgcccaggtgctgacgaccgacgcgggggtc cgcgaagccgaacgcgcccagttactaccgtggaccgcgacgctgaaacgtcactg tgtgcgggtgatcccgtgggagatcaccggccgccacttcaggttcggtccggaac cggaccgcagccagacctttgcctgcgaggcctcgtcacacaaccagcga

Table 2. (continued) DNA sequences of selected low oxygen induced antigens		
Rv no.	**SEQ ID NO:**	**Sequence**
Rv3130c	**84**	atgaatcacctaacgacacttgacgccgggtttctcaaggcagaagacgtggatcg gcacgtgagtctggcaatcggcgctctggcggtcatcgaggggccggctcccgatc aggaagccttcttatcgtcgctcgctcaacgcctacgtccctgtacccggttcggg cagcggttacgcctgcgcccgttcgacctcggtgcacccaaatgggtggacgatcc cgacttcgatcttggccgtcatgtgtggcgcatcgccttgccgcggcctggcaacg aagaccagttattcgagctgatcgccgatctgatggcgcgtcgtttggaccggggt cgaccgctgtgggaggtctgggtcatcgaaggcctggcggacagcaagtgggcgat cctgaccaaactgcaccactgcatggccgacggaatcgcggcgactcacctgctag ctgggctctccgatgaaagtatgagcgacagcttcgcgagcaacatccacacgacc atgcagtcgcaatccgcatctgtgcggcggggtggattccgtgtcaatccaagcga ggcgttgaccgcgtcgaccgccgtgatggcaggcatcgttcgcgcggccaagggtg ccagtgagatcgcggccggcgtgctaagtcccgccgcgtcgtcgttgaacgggccg atcagtgatttgcgtcgctacagcgcagcaaaggtccctctcgccgacgtcgaaca ggtgtgccggaaattcgacgtcaccatcaatgatgttgcgcttgccgcgattacgg aaagctaccgcaacgtcctcatccagcggggtgagcggcctaggtttgattcgctg cgtacgctagtgccggtctcgacgcgttccaacagcgctttgagcaagaccgataa ccgtgtttcgttaatgctgcccaacctgccggtggatcaagagaacccgctgcagc ggctgcggatcgtgcactcgcggctgactcgggccaaggcgggggacagagacaa ttcggaaatactttgatggcgattgccaaccgccttccgttccccatgaccgcatg ggcggtcgggctgttgatgcggctgccgcagcgtggtgttgtcaccgtggcgacaa atgtgccgggtccacgacggccgctgcagattatgggcagacgggtgcttgaccta tacccggtttcgccgatcgcgatgcaactgcgcaccagtgtcgcgatgctcagcta cgccgacgacctgtacttcgggatcctggccgactacgacgtggtagcagatgccg gccagctggcgcgaggaattgaagacgccgtcgcacggctggtggcgatcagtaag cggcgcaaggtgactcgcaggcgcggagcgctatcgctggttgtg
Rv3131	**85**	atgaacacccatttcccggacgccgaaaccgtgcgaacggttctcaccctggccgt ccgggcccctccatccacaacacgcagccgtggcggtggcgggtatgcccgacga gtctggagctgttctctagacccgatatgcagctgcgtagcaccgatccggacggg cgtgagttgatcctcagctgtggtgtggcattgcaccactgcgtcgtcgctttggc gtcgctgggctggcaggccaaggtaaaccgtttccccgatcccaaggaccgctgcc atctggccaccatcggggtacaaccgcttgttcccgatcaggccgatgtcgccttg gcggcggccataccgcggcgacgcaccgatcggcgcgcctacagttgctggccggt gccaggaggtgacatcgcgttgatggccgcaagagcagcccgtggcggggtcatgc tgcggcaggtcagtgccctagaccgaatgaaagccattgtggcgcaggctgtcttg gaccacgtgaccgacgaggaatatctgcgcgagctcaccatttggagtgggcgcta cggttcagtggccggggttcccgcccgcaacgagccgccatcagaccccagtgccc cgatccccggtcgcctgttcgccgggcccggtctgtctcagccgtccgacgtctta cccgctgacgacggcgccgcgatcctggcactaggcaccgagacagacgaccggtt ggcccggctgcgcgccggcgaggccgccagcatcgtcttgttgaccgcgacggcaa tggggctggcgtgctgcccgatcaccgaaccgctggagatcgccaagacccgcgac gcggtccgtgccgaggtgttcggcgccggcggctacccccagatgctgctgcgagt gggttgggcaccgatcaatgccgacccgttgccaccgacgccacggcgcgaactgt cccaggtcgttgagtggccggaagagctactgcgacaacggtgc

Table 2. (continued) DNA sequences of selected low oxygen induced antigens		
Rv no.	**SEQ ID NO:**	**Sequence**
Rv3132c	**86**	atgacaacaggggcctcgtcgacgaaaacgacggcgccgcaatgcgtccactgcg tcacacgctctcccaactacgcctgcacgagctgctggtcgaggtgcaggaccggg tcgagcagatcgtcgagggccgggaccgcctcgatggtctggtggaggccatgctc gtggtcacagcgggcctggacctggaggcaaccctacgcgctatcgtgcattcagc gaccagccttgtcgatgcgcgctatggcgctatggaggtgcacgaccggcagcatc gggtattgcactttgtctatgaaggcatcgacgaggagaccgttcggcggatcggc cacctaccgaaaggcctaggcgtcatcgggctgctcatcgaagatcccaaaccgtt acggctggacgatgtttctgcgcacccggcctcgattggttttccgccgtatcatc cgccgatgcgtaccttcctcggggtaccggttcgggtgcgcgatgaatcgttcggc actctgtacctgactgacaagaccaacgggcaaccgttcagcgacgacgacgaggt tctggtccaggcgctggcggccgccgcgggtatcgcagtcgcgaatgcccggctct accagcaggctaaggcgcgtcagtcgtggatcgaggccacccgtgacatcgccacc gagttgttgtccggcaccgaacccgcgacggtgttccggcttgtcgccgcggaggc gctcaagctgacggcggctgacgctgccctggtagccgttcccgtcgacgaggaca tgcctgccgctgacgtgggggagctgctggtgattgaaacagtcggcagcgctgtg gcttccattgttgggcgaacgattccggtggcgggcgcggtgctgcgggaggtctt cgtcaacggcattccgcgacgggtcgaccgggtcgatttggaaggcctggacgaac tggccgacgcaggtccggcgctgctgttgccgctgcgggccagaggtaccgtagcg ggtgtcgttgttgtgctgagtcaaggcggtccaggggctttcaccgacgaacaact cgagatgatggccgcgttcgccgaccaggccgcgctggcttggcaattggccactt cgcaacgtcggatgcgcgaactcgacgtactgaccgaccgggatcgtatcgcccgt gacctccatgaccatgtcatccagcggctcttcgcgattggcctggctttgcaggg tgctgtcccgcacgaacgtaatcctgaagtgcagcaacgactctcggacgtggtag acgatctgcaagacgttatacaggaaatccggaccaccatttatgacctgcacgga gcatcgcagggtatcactcggctccggcagcgaatcgatgcggccgtagcccaatt tgccgactcggggttgcgcaccagcgttcaattcgtgggtccattgtcggtggtcg acagcgcgctcgccgatcaggccgaggcggtggttcgggaagcggtcagcaacgcg gttcgccatgcgaaggccagcacgttgaccgtccgggtcaaagtcgacgacgactt gtgcatcgaggtgaccgacaacggccgcgggctgcccgacgagttcaccggaagcg gcttaacgaacctgcggcagcgggcagagcaggccggcggcgaattcaccctcgcg agcgtaccgggcgcgagcggaacagtgctgcgatggtcagcaccgttgtcgcag
Rv3134c	**87**	atgagcgatcctcggccagctcgggcagtggtcgttggtatcgacgggtcaagggc ggcaacgcatgcggcgttgtgggcggtcgatgaggcggtgaaccgagacattccgc tgcgactggtgtacgtcatcgatccgtcccaactgtccgccgccggcgagggcggt gggcaatcagcggcccgagcggcgctgcacgacgcctctcggaaggtcgaggccac cgggcaaccggtcaagatcgaaacggaggttctgtgcggcaggccgctcaccaagc tgatgcaggagtccaggtccgcggcgatgctgtgcgtcggttcggtggggcttgat catgtccgcggtcgccggggttcggtcgcggcgaccctggctgggtcggccttatg ccccgtggcggtgattcacccgtcgccggccgagccagcgacaacctcccaggtca gcgcggttgtcgcggaggtggacaatggtgtggtgctgcggcacgcattcgaggag gccaggctgcgcggagttccgctgcgggccgtggctgtccacgctgctgaaacacc cgatgacgtcgaacagggcagccggttggcgcatgtacacctgagccgtcggctcg cccactggacccggctctaccccgaggtgcgggtggatcgggccatcgccggcggc agtgcgtgccgtcatctggccgccaacgcaaagccgggtcagctgttcgtcgcgga ctcacactccgcgcacgaattgtgcggtgcataccagcccggatgcgccgtactta cggtacgcagtgccaacttg

Table 2. (continued) DNA sequences of selected low oxygen induced antigens		
Rv no.	**SEQ ID NO:**	**Sequence**
Rv3841	**88**	atgacagaatacgaagggcctaagacaaaattccacgcgttaatgcaggaacagat tcataacgaattcacagcggcacaacaatatgtcgcgatcgcggtttatttcgaca gcgaagacctgccgcagttggcgaagcatttttacagccaagcggtcgaggaacga aaccatgcaatgatgctcgtgcaacacctgctcgaccgcgaccttcgtgtcgaaat tcccggcgtagacacggtgcgaaaccagttcgacagaccccgcgaggcactggcgc tggcgctcgatcaggaacgcacagtcaccgaccaggtcggtcggctgacagcggtg gcccgcgacgagggcgatttcctcggcgagcagttcatgcagtggttcttgcagga acagatcgaagaggtggccttgatggcaaccctggtgcgggttgccgatcgggccg gggccaacctgttcgagctagagaacttcgtcgcacgtgaagtggatgtggcgccg gccgcatcaggcgccccgcacgctgccgggggccgcctc
Rv3842c	**89**	atgacatgggccgacgaggtgctcgccggacatcctttgtggttgctcaccgtgg tgcgtcggcggctcggccggagcataccttgccgcctacgacctggcgctcaaag agggcgccgacggcgtggaatgtgatgtgcggttgacccgggacgggcatctggtc tgtgtgcatgaccgccgcctggaccgaacctcgacgggagccggcttggtcagcac gatgacgctggcccagctacgcgagctggagtacggcgcgtggcacgacagctggc gccccgacggttcgcacggcgacaccagtctgctgaccctggacgcgcttgtttcg ctggttttggactggcaccggccggtgaagatcttcgtcgagaccaagcatcccgt ccgatacggctcgctggtggaaaacaagctgctggcgctgctacaccggttcggta ttgccgcacccgcctccgcagatcgatcccgtgcggtggtgatgtcgttttcggcc gccgcggtctggcggatccggcgggctgcaccgctgctgccgacggtgttgctcgg caagacccccgatacctgaccagcagtgcggccacggcggtcggggcaaccgccg tgggaccctcactgcctgcgttaaaggaatatccgcaactcgttgaccgctcggca gctcagggccgggcggtgtactgctggaacgtcgatgagtacgaggacatcgactt ttgccgggaggtcggggtggcctggattggtactcaccaccccggccgcaccaagg cctggctggaagacgggcgggcgaacgggaccactcgc
Rv3908	**90**	gtgtccgacggcgaacaagccaaatcacgtcgacgccgggggcggcgccgcgggcg gcgcgctgcggctacagccgagaatcacatggacgcccaaccggccggcgacgcca ccccgaccccggcaacggcgaagcggtcccggtcccgctcacctcgtcgcgggtcg actcggatgcgcaccgtgcacgaaacatcggctggagggttggtcattgacggtat cgacggtccacgagacgcgcaggtcgcggctctgatcggccgcgtcgaccggcgcg gccggctgctgtggtcgctacccaaggggcacatcgagttgggcgagaccgccgag cagaccgccatccgcgaggtcgccgaggagaccggcatccgcggcagtgtgctcgc cgcgctggggcgcatcgactactggttcgtcaccgacggccggcgggtgcacaaga ccgtccaccattatttgatgcggttttaggcggagagctgtccgacgaagacctc gaggtagccgaggtagcctgggtgccgatccgggaactgccgtctcgactggccta cgccgacgaacgtcgactagccgaggtggccgacgaactgatcgacaagctgcaga gcgacggccccgccgcgcttccgccgctaccacccagctcgcctcgtcgacggccg caaacgcattcacgcgctcgtcatgccgatgactcagcaccgggtcagcacaacgg tcccgggccggggccg

Preferably the immunogenic portions are selected from the group consisting of the sequences presented in Table 1 and the nucleic acid sequences are selected from the sequences presented in Table 2.

In another embodiment, the vaccine is a multiphase vaccine, where the polypeptides or fragments hereof are fused to other antigens with efficacy as prophylactic vaccines, where the fusion partner is selected from e.g. the group consisting of ESAT-6,

TB10.4, CFP10, RD1-ORF5, RD1-ORF2, Rv1036, MPB64, MPT64, Ag85A, Ag85B (MPT59), MPB59, Ag85C, 19kDa lipoprotein, MPT32.

The invention further discloses a therapeutic vaccine against tuberculosis comprising one or more polypeptides or fragments hereof, which polypeptides are expressed during the latent stage of the mycobacteria infection, which stage is characterized by low-oxygen tension in the microenvironment of the mycobacteria, or nucleic acids encoding these polypeptides.

Preferably, the therapeutic and multiphase vaccine comprises an additional delivery system selected from among, live recombinant vaccines, that is gene-modified organisms such as bacteria or viruses expressing mycobacteria genes, or immunogenic delivery systems such as, DNA vaccines, that is plasmids expressing genes or gene fragments for the proteins described above, or protein vaccines, that is the proteins themselves or synthetic peptides derived from the proteins themselves delivered in a delivery system such as an adjuvant.

The invention further discloses a therapeutic vaccine in which the amino acid sequence is lipidated so as to allow a self-adjuvanting effect of the polypeptide.

The invention also discloses a method for treating an animal, including a human being, with tuberculosis caused by virulent mycobacteria, e.g., by *Mycobacterium tuberculosis*, *Mycobacterium africanum* or *Mycobacterium bovis*, comprising administering to the animal the above-mentioned vaccine.

The invention also discloses a method for immunizing an animal, including a human being, against tuberculosis caused by virulent mycobacteria, e.g., by *Mycobacterium tuberculosis*, *Mycobacterium africanum* or *Mycobacterium bovis*, comprising administering to the animal the above mentioned vaccine.

In a still further embodiment, the invention discloses an immunogenic composition comprising a polypeptide as defined above, preferably in the form of a vaccine or in the form of a diagnostic reagent. The diagnostic reagent can be in the form of a skin test reagent (administered by the transcutaneous, subcutaneous or intradermal routes), a serological reagent or a reagent for stimulating a cell-mediated reaction.

In another embodiment, the invention discloses a nucleic acid fragment in isolated form which

(a) comprises a nucleic acid sequence which encodes a polypeptide as defined above, or comprises a nucleic acid sequence complementary thereto; or

(b) has a length of at least 10 nucleotides and hybridizes readily under stringent hybridization conditions with a nucleotide sequence selected from the nucleotide sequences presented in Table 2 or a sequence complementary thereto, or with a nucleotide sequence selected from a sequence in (a)

The nucleic acid fragment is preferably a DNA fragment. The fragment can be used as a pharmaceutical.

In another embodiment, the invention discloses a vaccine comprising a nucleic acid fragment according to the invention, optionally inserted in a vector, the vaccine effecting *in vivo* expression of antigen by a human being or other mammal or animal, to whom the vaccine has been administered, the amount of expressed antigen being effective to confer substantially increased resistance to tuberculosis caused by virulent mycobacteria, e.g. by *Mycobacterium tuberculosis*, *Mycobacterium africanum* or *Mycobacterium bovis,* in an animal, including a human being.

In a further embodiment, the invention discloses the use of a nucleic acid fragment according to the invention for the preparation of a composition for the diagnosis of tuberculosis caused by virulent mycobacteria, e. g., by *Mycobacterium tuberculosis*, *Mycobacterium africanum* or *Mycobacterium bovis*, and the use of a nucleic acid fragment according to the invention for the preparation of a pharmaceutical composition for the vaccination against tuberculosis caused by virulent mycobacteria, e.g., by *Mycobacterium tuberculosis*, *Mycobacterium africanum* or *Mycobacterium bovis.*

In a still further embodiment, the invention discloses a vaccine for immunizing an human being or other mammal or animal, against tuberculosis caused by virulent mycobacteria, e.g. by *Mycobacterium tuberculosis*, *Mycobacterium africanum* or *Mycobacterium bovis*, comprising as the effective component a non-pathogenic microorganism, wherein at least one copy of a DNA fragment comprising a DNA sequence

encoding a polypeptide as defined above has been incorporated into the microorganism (e.g., placed on a plasmid or in the genome) in a manner allowing the microorganism to express and optionally secrete the polypeptide.

In another embodiment, the invention discloses a replicable expression vector, which comprises a nucleic acid fragment according to the invention, and a transformed cell harboring at least one such vector.

In another embodiment, the invention discloses a method for producing a polypeptide as defined above, comprising

(a) inserting a nucleic acid fragment according to the invention into a vector that is able to replicate in a host cell, introducing the resulting recombinant vector into the host cell, culturing the host cell in a culture medium under conditions sufficient to effect expression of the polypeptide, and recovering the polypeptide from the host cell or culture medium;

(b) isolating the polypeptide from a whole mycobacterium, e.g. *Mycobacterium tuberculosis*, *Mycobacterium africanum* or *Mycobacterium bovis*, from culture filtrate or from lysates or fractions thereof; or

(c) synthesizing the polypeptide e.g. by solid or liquid phase peptide synthesis.

The invention also discloses a method of diagnosing tuberculosis caused by virulent mycobacteria, e.g. by *Mycobacterium tuberculosis*, *Mycobacterium africanum* or *Mycobacterium bovis,* in an animal, including a human being, comprising intradermally injecting, in the animal, a polypeptide as defined above or an immunogenic composition as defined above, a positive skin response at the location of injection being indicative of the animal having tuberculosis, and a negative skin response at the location of injection being indicative of the animal not having tuberculosis.

In another embodiment, the invention discloses a method for immunizing an animal, including a human being, against tuberculosis caused by virulent mycobacteria, e.g. by *Mycobacterium tuberculosis*, *Mycobacterium africanum* or *Mycobacterium bovis*, comprising administering to the animal the polypeptide as defined above, the immunogenic composition according to the invention, or the vaccine according to the invention.

Another embodiment of the invention discloses a monoclonal or polyclonal antibody, which is specifically reacting with a polypeptide as defined above in an immuno assay, or a specific binding fragment of said antibody. Preferably, said antibody is for use as a diagnostic reagent, e.g. for detection of mycobacteria antigens in sputum, urine or other body fluids of an infected animal, including a human being.

In a further embodiment the invention discloses a pharmaceutical composition that comprises an immunologically responsive amount of at least one member selected from the group consisting of:

(a) a polypeptide selected from the sequences presented in Table 1, or an immunogenic portion thereof;

(b) an amino acid sequence which has a sequence identity of at least 70% to any one of said polypeptides in (a) and is immunogenic;

(c) a fusion polypeptide comprising at least one polypeptide or amino acid sequence according to (a) or (b) and at least one fusion partner;

(d) a nucleic acid sequence which encodes a polypeptide or amino acid sequence according to (a), (b) or (c);

(e) a nucleic acid sequence which is complementary to a sequence according to (d);

(f) a nucleic acid sequence which has a length of at least 10 nucleotides and which hybridizes under stringent conditions with a nucleic acid sequence according to (d) or (e); and

(g) a non-pathogenic micro-organism which has incorporated (e.g. placed on a plasmid or in the genome) therein a nucleic acid sequence according to (d), (e) or (f) in a manner to permit expression of a polypeptide encoded thereby.

In a still further embodiment the invention discloses a method for stimulating an immunogenic response in an animal which comprises administering to said animal an immunologically stimulating amount of at least one member selected from the group consisting of:

(a) a polypeptide selected from the sequences presented in Table 1, or an immunogenic portion thereof;

(b) an amino acid sequence which has a sequence identity of at least 70% to any one of said polypeptides in (a) and is immunogenic;

(c) a fusion polypeptide comprising at least one polypeptide or amino acid sequence according to (a) or (b) and at least one fusion partner;

(d) a nucleic acid sequence which encodes a polypeptide or amino acid sequence according to (a), (b) or (c);

(e) a nucleic acid sequence that is complementary to a sequence according to (d);

(f) a nucleic acid sequence which has a length of at least 10 nucleotides and which hybridizes under stringent conditions with a nucleic acid sequence according to (d) or (e); and

(g) a non-pathogenic micro-organism which has incorporated therein (e.g. placed on a plasmid or in the genome) a nucleic acid sequence according to (d), (e) or (f) in a manner to permit expression of a polypeptide encoded thereby.

The vaccine, immunogenic composition and pharmaceutical composition according to the invention can be used therapeutically in a subject infected with a virulent mycobacterium combined with a prophylactic composition in a subject to prevent further infection with a virulent mycobacterium.

The invention also discloses a method for diagnosing previous or ongoing infection with a virulent mycobacterium, said method comprising

(a) contacting a sample, e.g. a blood sample, with a composition comprising an antibody according to the invention, a nucleic acid fragment according to the invention and/or a polypeptide as defined above, or

(b) contacting a sample, e.g. a blood sample comprising mononuclear cells (e.g. T-lymphocytes), with a composition comprising one or more polypeptides as defined above in order to detect a positive reaction, e.g. proliferation of the cells or release of cytokines such as IFN-γ.

Finally, the invention discloses a method of diagnosing *Mycobacterium tuberculosis* infection in a subject comprising:

(a) contacting a polypeptide as defined above with a bodily fluid of the subject;

(b) detecting binding of a antibody to said polypeptide, said binding being an indication that said subject is infected by *Mycobacterium tuberculosis* or is susceptible to *Mycobacterium tuberculosis* infection.

Definitions

Polypeptides

The word "polypeptide" in the present invention should have its usual meaning. That is an amino acid chain of any length, including a full-length protein, oligopeptides, short peptides and fragments thereof, wherein the amino acid residues are linked by covalent peptide bonds.

The polypeptide may be chemically modified by being glycosylated, by being lipidated (e.g. by chemical lipidation with palmitoyloxy succinimide as described by Mowat et al. 1991 or with dodecanoyl chloride as described by Lustig et al. 1976), by comprising prosthetic groups, or by containing additional amino acids such as e.g. a his-tag or a signal peptide.

Each polypeptide may thus be characterized by specific amino acids and be encoded by specific nucleic acid sequences. It will be understood that such sequences include analogues and variants produced by recombinant or synthetic methods wherein such polypeptide sequences have been modified by substitution, insertion, addition or deletion of one or more amino acid residues in the recombinant polypeptide and still be immunogenic in any of the biological assays described herein. Substitutions are preferably "conservative". These are defined according to the following table. Amino acids in the same block in the second column and preferably in the same line in the third column may be substituted for each other. The amino acids in the third column are indicated in one-letter code.

ALIPHATIC	Non-polar	GAP
		ILV
	Polar-uncharged	CSTM
		NQ
	Polar-charged	DE
		KR
AROMATIC		HFWY

A preferred polypeptide within the present invention is an immunogenic antigen from *M. tuberculosis* produced when the organism is subjected to the stresses associated with latent infection. Such antigen can for example also be derived from the *M. tuberculosis* cell and/or *M. tuberculosis* culture filtrate. Thus, a polypeptide comprising an immunogenic portion of one of the above antigens may consist entirely of the immunogenic portion, or may contain additional sequences. The additional sequences may be derived from the native *M. tuberculosis* antigen or be heterologous and such sequences may, but need not, be immunogenic.

Each polypeptide is encoded by a specific nucleic acid sequence. It will be understood that such sequences include analogues and variants hereof wherein such nucleic acid sequences have been modified by substitution, insertion, addition or deletion of one or more nucleic acids. Substitutions are preferably silent substitutions in the codon usage that will not lead to any change in the amino acid sequence, but may be introduced to enhance the expression of the protein.

In the present context the term "substantially pure polypeptide fragment" means a polypeptide preparation which contains at most 5% by weight of other polypeptide material with which it is natively associated (lower percentages of other polypeptide material are preferred, e.g. at most 4%, at most 3%, at most 2%, at most 1%, and at most ½%). It is preferred that the substantially pure polypeptide is at least 96% pure, *i.e.* that the polypeptide constitutes at least 96% by weight of total polypeptide material present in the preparation, and higher percentages are preferred, such as at least 97%, at least 98%, at least 99%, at least 99.25%, at least 99.5%, and at least 99,75%. It is especially preferred that the polypeptide fragment is in "essentially pure form", *i.e.* that the polypeptide fragment is essentially free of any other antigen with which it is natively associated, *i.e.* free of any other antigen from bacteria belonging to the tuberculosis complex or a virulent mycobacterium. This can be accomplished by preparing the polypeptide fragment by means of recombinant methods in a non-mycobacterial host cell as will be described in detail below, or by synthesizing the polypeptide fragment by the well-known methods of solid or liquid phase peptide synthesis, e.g. by the method described by Merrifield or variations thereof.

By the term "virulent mycobacterium" is understood a bacterium capable of causing the tuberculosis disease in an animal or in a human being. Examples of virulent my-

cobacteria include but are not limited to *M. tuberculosis, M. africanum,* and *M. bovis.* Examples of relevant animals are cattle, possums, badgers and kangaroos.

By "a TB patient" is understood an individual with culture or microscopically proven infection with virulent mycobacteria, and/or an individual clinically diagnosed with TB and who is responsive to anti-TB chemotherapy. Culture, microscopy and clinical diagnosis of TB are well known by any person skilled in the art.

By the term "PPD-positive individual" is understood an individual with a positive Mantoux test or an individual where PPD induces a positive *in vitro* recall response determined by release of IFN-γ.

By "a latently infected individual" is understood an individual, who has been infected by a virulent mycobacterium, e.g. *M. tuberculosis* , but shows no sign of active tuberculosis. It is likely that individuals who have been vaccinated, e.g. by BCG, or treated for TB may still retain the mycobacteria within their bodies, although this is currently impossible to prove since such individuals would be expected to be positive if tested for PPD reactivity. Nonetheless, in its most accurate sense, "latently-infected" may be used to describe any individual who has *M. tuberculosis* residing in their tissues but who is not clinically ill.

By the term "delayed type hypersensitivity reaction" (DTH) is understood a T-cell mediated inflammatory response elicited after the injection of a polypeptide into, or application to, the skin, said inflammatory response appearing 72-96 hours after the polypeptide injection or application.

By the term "IFN-γ" is understood interferon-gamma. The measurement of IFN-γ is used as an indication of an immunological response.

By the terms "nucleic acid fragment" and "nucleic acid sequence" are understood any nucleic acid molecule including DNA, RNA , LNA (locked nucleic acids), PNA, RNA, dsRNA and RNA-DNA-hybrids. Also included are nucleic acid molecules comprising non-naturally occurring nucleosides. The term includes nucleic acid molecules of any length e.g. from 10 to 10000 nucleotides, depending on the use. When the nucleic

acid molecule is for use as a pharmaceutical, e.g. in DNA therapy, or for use in a method for producing a polypeptide according to the invention, a molecule encoding at least one epitope is preferably used, having a length from about 18 to about 1000 nucleotides, the molecule being optionally inserted into a vector. When the nucleic acid molecule is used as a probe, as a primer or in antisense therapy, a molecule having a length of 10-100 is preferably used. According to the invention, other molecule lengths can be used, for instance a molecule having at least 12, 15, 21, 24, 27, 30, 33, 36, 39, 42, 50, 60, 70, 80, 90, 100, 200, 300, 400, 500 or 1000 nucleotides (or nucleotide derivatives), or a molecule having at most 10000, 5000, 4000, 3000, 2000, 1000, 700, 500, 400, 300, 200, 100, 50, 40, 30 or 20 nucleotides (or nucleotide derivatives).

The term "stringent" when used in conjunction with hybridization conditions is as defined in the art, i.e. the hybridization is performed at a temperature not more than 15-20°C under the melting point T_m, cf. Sambrook et al, 1989, pages 11.45-11.49. Preferably, the conditions are "highly stringent", i.e. 5-10°C under the melting point T_m.

Throughout this specification, unless the context requires otherwise, the word "comprise", or variations thereof such as "comprises" or "comprising", will be understood to imply the inclusion of a stated element or integer or group of elements or integers but not the exclusion of any other element or integer or group of elements or integers.

Sequence identity

The term "sequence identity" indicates a quantitative measure of the degree of homology between two amino acid sequences of equal length or between two nucleotide sequences of equal length. The two sequences to be compared must be aligned to best possible fit allowing the insertion of gaps or alternatively, truncation at the ends of the protein sequences. The sequence identity can be calculated as $\frac{(N_{ref}-N_{dif})100}{N_{ref}}$, wherein N_{dif} is the total number of non-identical residues in the two sequences when aligned and wherein N_{ref} is the number of residues in one of the sequences. Hence, the DNA sequence AGTCAGTC [SEQ ID NO: 184] will have a sequence identity of 75% with the sequence AATCAATC, SEQ ID NO: 185 (N_{dif}=2 and N_{ref}=8). A gap is counted as non-identity of the specific residue(s), i.e. the DNA sequence AGTGTC [SEQ ID NO: 186] will have a sequence identity of 75% with the

DNA sequence AGTCAGTC, SEQ ID NO: 187, (N_{dif}=2 and N_{ref}=8). Sequence identity can alternatively be calculated by the BLAST program e.g. the BLASTP program (Pearson, 1988, or www.ncbi.nlm.nih.gov/cgi-bin/BLAST). In one aspect of the invention, alignment is performed with the sequence alignment method ClustalW with default parameters as described by Thompson J., *et al* 1994, available at http://www2.ebi.ac.uk/clustalw/.

A preferred minimum percentage of sequence identity is at least 80%, such as at least 85%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, and at least 99.5%.

Immunogenic portion

In a preferred embodiment of the invention, the polypeptide comprises an immunogenic portion of the polypeptide, such as an epitope for a B-cell or T-cell.

The immunogenic portion of a polypeptide is a part of the polypeptide, which elicits an immune response in an animal or a human being, and/or in a biological sample determined by any of the biological assays described herein. The immunogenic portion of a polypeptide may be a T-cell epitope or a B-cell epitope. Immunogenic portions can be related to one or a few relatively small parts of the polypeptide, they can be scattered throughout the polypeptide sequence or be situated in specific parts of the polypeptide. For a few polypeptides, epitopes have even been demonstrated to be scattered throughout the polypeptide covering the full sequence (Ravn et al 1999). In order to identify relevant T-cell epitopes which are recognized during an immune response, it is possible to use overlapping oligopeptides for the detection of MHC class II epitopes, preferably synthetic, having a length of e.g. 20 amino acid residues derived from the polypeptide. These peptides can be tested in biological assays (e.g. the IFN-γ assay as described herein) and some of these will give a positive response (and thereby be immunogenic) as evidence for the presence of a T cell epitope in the peptide. For the detection of MHC class I epitopes it is possible to predict peptides that will bind (Stryhn et al. 1996) and hereafter produce these peptides synthetic and test them in relevant biological assays, e.g.. the IFN-γ assay as described herein. The peptides preferably having a length of, e.g., 8 to 11 amino acid residues derived from the polypeptide. B-cell epitopes can be determined by analyzing the B cell recognition to overlapping peptides covering the polypeptide of interest as, e.g., described in Harboe et al 1998.

Although the minimum length of a T-cell epitope has been shown to be at least 6 amino acids, it is normal that such epitopes are constituted of longer stretches of amino acids. Hence, it is preferred that the polypeptide fragment of the invention has a length of at least 7 amino acid residues, such as at least 8, at least 9, at least 10, at least 12, at least 14, at least 16, at least 18, at least 20, at least 22, at least 24, and at least 30 amino acid residues. Hence, in important embodiments of the inventive method, it is preferred that the polypeptide fragment has a length of at most 50 amino acid residues, such as at most 40, 35, 30, 25, and 20 amino acid residues. It is expected that the peptides having a length of from 10 to 20 amino acid residues will prove to be most efficient as MHC class II epitopes and therefore especially preferred lengths of the polypeptide fragment used in the inventive method are 18, such as 15, 14, 13, 12 and even 11 amino acid residues. It is expected that the peptides having a length of from 7 to 12 amino acid residues will prove to be most efficient as MHC class I epitopes and therefore especially preferred lengths of the polypeptide fragment used in the inventive method are 11, such as 10, 9, 8 and even 7 amino acid residues.

Immunogenic portions of polypeptides may be recognized by a broad part (high frequency) or by a minor part (low frequency) of the genetically heterogeneous human population. In addition some immunogenic portions induce high immunological responses (dominant), whereas others induce lower, but still significant, responses (subdominant). High frequency><low frequency can be related to the immunogenic portion binding to widely distributed MHC molecules (HLA type) or even by multiple MHC molecules (Sinigaglia, 1988, Kilgus, 1991).

In the context of providing candidate molecules for a new vaccine against tuberculosis, the subdominant epitopes are however as relevant as are the dominant epitopes since it has been shown (Olsen, 2000) that such epitopes can induce protection regardless of the fact that they are not as strongly or broadly recognized.

Variants

A common feature of the polypeptides of the invention is their capability to induce an immunological response as illustrated in the examples. It is understood that a variant

of a polypeptide of the invention produced by substitution, insertion, addition or deletion may also be immunogenic as determined by any of the assays described herein.

Immune individual

An immune individual is defined as a person or an animal, which has cleared or controlled an infection with virulent mycobacteria or has received a vaccination with *M.bovis* BCG.

Immune response

The immune response may be monitored by one of the following methods:

- An in vitro cellular response is determined by induction of the release of a relevant cytokine such as IFN-γ from, or the induction of proliferation in lymphocytes withdrawn from an animal or human being currently or previously infected with virulent mycobacteria or immunized with the relevant polypeptide. The induction being performed by the addition of the polypeptide or the immunogenic portion of the polypeptide to a suspension comprising from $2x10^5$ cells to $4x10^5$ cells per well. The cells being isolated from either the blood, the spleen, the liver or the lung and the addition of the polypeptide or the immunogenic portion resulting in a concentration of not more than 20 μg per ml suspension and the stimulation being performed from two to five days. For monitoring cell proliferation the cells are pulsed with radioactive labeled Thymidine and after 16-22 hours of incubation detecting the proliferation by liquid scintillation counting. A positive response is defined as being a response more than background plus two standard deviations. The release of IFN-γ can be determined by the ELISA method, which is well known to a person skilled in the art. A positive response being a response more than background plus two standard deviations. Other cytokines than IFN-γ could be relevant when monitoring the immunological response to the polypeptide, such as IL-12, TNF-α, IL-4, IL-5, IL-10, IL-6, TGF-β. Another and more sensitive method for detecting the immune response is the ELISpot method, in which the frequency of IFN-γ producing cells is determined. In an ELIspot plate (MAHA, Millipore) precoated with anti-murine IFN-γ antibodies (PharMingen) graded numbers of cells isolated from either blood, spleen, or lung

(typically between 1 to 4 x 10^5 cells /well) are incubated for 24-32 hrs in the presence of the polypeptide or the immunogenic portion resulting in a concentration of not more than 20 μg per ml. The plates are subsequently incubated with biotinylated anti-IFN-γ antibodies followed by a streptavidin-alkaline phosphatase incubation. The IFN-γ producing cells are identified by adding BCIP/NBT (Sigma), the relevant substrate giving rise to spots. These spots can be enumerated using a dissection microscope. It is also a possibility to determine the presence of mRNA coding for the relevant cytokine by the use of the PCR technique. Usually one or more cytokines will be measured utilizing for example PCR, ELISPOT or ELISA. It will be appreciated by a person skilled in the art that a significant increase or decrease in the amount of any of these cytokines induced by a specific polypeptide can be used in evaluation of the immunological activity of the polypeptide.

- An *in vitro* cellular response may also be determined by the use of T cell lines derived from an immune individual or an *M. tuberculosis*-infected person where the T cell lines have been driven with either live mycobacteria, extracts from the bacterial cell or culture filtrate for 10 to 20 days with the addition of IL-2. The induction being performed by addition of not more than 20 μg polypeptide per ml suspension to the T cell lines containing from 1x10^5 cells to 3x10^5 cells per well and incubation being performed from two to six days. The induction of IFN-γ or release of another relevant cytokine is detected by ELISA. The stimulation of T cells can also be monitored by detecting cell proliferation using radioactively labeled Thymidine as described above. For both assays a positive response being a response more than background plus two standard deviations.

- An in *vivo* cellular response may be determined as a positive DTH response after intradermal injection or local application patch of at most 100 μg of the polypeptide or the immunogenic portion to an individual who is clinically or subclinically infected with a virulent mycobacterium, a positive response having a diameter of at least 5 mm 72-96 hours after the injection or application.

- An *in vitro* humoral response is determined by a specific antibody response in an immune or infected individual. The presence of antibodies may be determined by an ELISA technique or a Western blot where the polypeptide or the immunogenic portion is absorbed to either a nitrocellulose membrane or a polystyrene surface. The serum is preferably diluted in PBS from 1:10 to 1:100 and added to the absorbed polypeptide and the incubation being performed from 1 to 12 hours. By the use of labeled secondary antibodies the presence of specific antibodies can be determined by measuring the OD e.g. by ELISA where a positive response is a response of more than background plus two standard deviations or alternatively a visual response in a Western blot.

- Another relevant parameter is measurement of the protection in animal models induced after vaccination with the polypeptide in an adjuvant or after DNA vaccination. Suitable animal models include primates, guinea pigs or mice, which are challenged with an infection of a virulent Mycobacterium. Readout for induced protection could be decrease of the bacterial load in target organs compared to non-vaccinated animals, prolonged survival times compared to non-vaccinated animals and diminished weight loss compared to non-vaccinated animals.

Preparation methods

In general, *M. tuberculosis* antigens, and DNA sequences encoding such antigens, may be prepared using any one of a variety of procedures.

They may be purified as native proteins from the *M. tuberculosis* cell or culture filtrate by procedures such as those described above. Immunogenic antigens may also be produced recombinantly using a DNA sequence encoding the antigen, which has been inserted into an expression vector and expressed in an appropriate host. Examples of host cells are *E. coli*. The polypeptides or immunogenic portion hereof can also be produced synthetically having fewer than about 100 amino acids, and generally fewer than 50 amino acids and may be generated using techniques well known

to those ordinarily skilled in the art, such as commercially available solid-phase techniques where amino acids are sequentially added to a growing amino acid chain.

In the construction and preparation of plasmid DNA encoding the polypeptide as defined for DNA vaccination a host strain such as *E. coli* can be used. Plasmid DNA can then be prepared from cultures of the host strain carrying the plasmid of interest, and purified using e.g. the Qiagen Giga -Plasmid column kit (Qiagen, Santa Clarita, CA, USA) including an endotoxin removal step. It is preferred that plasmid DNA used for DNA vaccination is endotoxin free.

Fusion proteins

The immunogenic polypeptides may also be produced as fusion proteins, by which methods superior characteristics of the polypeptide of the invention can be achieved. For instance, fusion partners that facilitate export of the polypeptide when produced recombinantly, fusion partners that facilitate purification of the polypeptide, and fusion partners which enhance the immunogenicity of the polypeptide fragment of the invention are all interesting possibilities. Therefore, the invention also pertains to a fusion polypeptide comprising at least one polypeptide or immunogenic portion defined above and at least one fusion partner. The fusion partner can, in order to enhance immunogenicity, be another polypeptide derived from *M. tuberculosis*, such as of a polypeptide fragment derived from a bacterium belonging to the tuberculosis complex, such as ESAT-6, TB10.4, CFP10, RD1-ORF5, RD1-ORF2, Rv1036, MPB64, MPT64, Ag85A, Ag85B (MPT59), MPB59, Ag85C, 19kDa lipoprotein, MPT32 and alpha-crystalline, or at least one T-cell epitope of any of the above mentioned antigens (Skjøt et al 2000; Danish Patent application PA 2000 00666; Danish Patent application PA 1999 01020; US patent application 09/0505,739; Rosenkrands *et al* 1998; Nagai et al 1991). The invention also pertains to a fusion polypeptide comprising mutual fusions of two or more of the polypeptides (or immunogenic portions thereof) of the invention.

Other fusion partners, which could enhance the immunogenicity of the product, are lymphokines such as IFN-γ, IL-2 and IL-12. In order to facilitate expression and/or purification, the fusion partner can e.g. be a bacterial fimbrial protein, e.g. the pilus components pilin and papA; protein A; the ZZ-peptide (ZZ-fusions are marketed by Pharmacia in Sweden); the maltose binding protein; glutathione S-transferase; β-

galactosidase; or poly-histidine. Fusion proteins can be produced recombinantly in a host cell, which could be *E. coli*, and it is a possibility to induce a linker region between the different fusion partners.

Other interesting fusion partners are polypeptides, which are lipidated so that the immunogenic polypeptide is presented in a suitable manner to the immune system. This effect is e.g. known from vaccines based on the *Borrelia burgdorferi* OspA polypeptide as described in e.g. WO 96/40718 A or vaccines based on the *Pseudomonas aeruginosa* OprI lipoprotein (Cote-Sierra J 1998). Another possibility is N-terminal fusion of a known signal sequence and an N-terminal cystein to the immunogenic polypeptide. Such a fusion results in lipidation of the immunogenic polypeptide at the N-terminal cystein, when produced in a suitable production host.

Uses

Protein Vaccine

Another part of the invention pertains to a vaccine composition comprising a polypeptide (or at least one immunogenic portion thereof) or fusion polypeptide according to the invention. In order to ensure optimum performance of such a vaccine composition it is preferred that it comprises an immunologically and pharmaceutically acceptable carrier, vehicle or adjuvant.

An effective vaccine, wherein a polypeptide of the invention is recognized by the animal, will in an animal model be able to decrease bacterial load in target organs, prolong survival times and/or diminish weight loss after challenge with a virulent Mycobacterium, compared to non-vaccinated animals

Suitable carriers are selected from the group consisting of a polymer to which the polypeptide(s) is/are bound by hydrophobic non-covalent interaction, such as a plastic, e.g. polystyrene, or a polymer to which the polypeptide(s) is/are covalently bound, such as a polysaccharide, or a polypeptide, e.g. bovine serum albumin, ovalbumin or keyhole limpet haemocyanin. Suitable vehicles are selected from the group consisting of a diluent and a suspending agent. The adjuvant is preferably selected from the group consisting of dimethyldioctadecylammonium bromide (DDA), Quil A, poly I:C, aluminum hydroxide, Freund's incomplete adjuvant, IFN-γ, IL-2, IL-12, mono-

phosphoryl lipid A (MPL), Trehalose Dimycolate (TDM), Trehalose Dibehenate and muramyl dipeptide (MDP).

Preparation of vaccines which contain peptide sequences as active ingredients is generally well understood in the art, as exemplified by U.S. Patents 4,608,251; 4,601,903; 4,599,231 and 4,599,230, all incorporated herein by reference.

Other methods of achieving adjuvant effect for the vaccine include use of agents such as aluminum hydroxide or phosphate (alum), synthetic polymers of sugars (Carbopol), aggregation of the protein in the vaccine by heat treatment, aggregation by reactivating with pepsin treated (Fab) antibodies to albumin, mixture with bacterial cells such as *C. parvum* or endotoxins or lipopolysaccharide components of gram-negative bacteria, emulsion in physiologically acceptable oil vehicles such as mannide mono-oleate (Aracel A) or emulsion with 20 percent solution of a perfluorocarbon (Fluosol-DA) used as a block substitute may also be employed. Other possibilities involve the use of immune modulating substances such as cytokines or synthetic IFN-γ inducers such as poly I:C in combination with the above-mentioned adjuvants.

Another interesting possibility for achieving adjuvant effect is to employ the technique described in Gosselin *et al.*, 1992 (which is hereby incorporated by reference herein). In brief, a relevant antigen such as an antigen of the present invention can be conjugated to an antibody (or antigen binding antibody fragment) against the Fcγ receptors on monocytes/macrophages.

The vaccines are administered in a manner compatible with the dosage formulation, and in such amount as will be therapeutically effective and immunogenic. The quantity to be administered depends on the subject to be treated, including, e.g., the capacity of the individual's immune system to mount an immune response, and the degree of protection desired. Suitable dosage ranges are of the order of several hundred micrograms active ingredient per vaccination with a preferred range from about 0.1 μg to 1000 μg, such as in the range from about 1 μg to 300 μg, and especially in the range from about 10 μg to 50 μg. Suitable regimens for initial administration and booster shots are also variable but are typified by an initial administration followed by subsequent inoculations or other administrations.

The manner of application may be varied widely. Any of the conventional methods for administration of a vaccine are applicable. These are believed to include oral application on a solid physiologically acceptable base or in a physiologically acceptable dispersion, parenterally, by injection or the like. The dosage of the vaccine will depend on the route of administration and will vary according to the age of the person to be vaccinated and, to a lesser degree, the size of the person to be vaccinated.

The vaccines are conventionally administered parenterally, by injection, for example, either subcutaneously or intramuscularly. Additional formulations which are suitable for other modes of administration include suppositories and, in some cases, oral formulations. For suppositories, traditional binders and carriers may include, for example, polyalkylene glycols or triglycerides; such suppositories may be formed from mixtures containing the active ingredient in the range of 0.5% to 10%, preferably 1-2%. Oral formulations include such normally employed excipients as, for example, pharmaceutical grades of mannitol, lactose, starch, magnesium stearate, sodium saccharine, cellulose, magnesium carbonate, and the like. These compositions take the form of solutions, suspensions, tablets, pills, capsules, sustained release formulations or powders and advantageously contain 10-95% of active ingredient, preferably 25-70%.

In many instances, it will be necessary to have multiple administrations of the vaccine. Especially, vaccines can be administered to prevent an infection with virulent mycobacteria, a prophylactic vaccine, and/or to treat established mycobacterial infection, a therapeutic vaccine. When administered to prevent an infection, the vaccine is given prophylactically, before definitive clinical signs, diagnosis or identification of an infection TB are present. Since the current vaccine BCG appears to induce an effective, but short-lived immune response, prophylactic vaccines may also be designed to be used as booster vaccines. Such booster vaccines are given to individuals who have previously received a vaccination, with the intention of prolonging the period of protection. In instances where the individual has already become infected or is suspected to have become infected, the previous vaccination may have provided sufficient immunity to prevent primary disease, but as discussed previously, boosting this immune response will not help against the latent infection. In such a situation, the vaccine will necessarily have to be a therapeutic vaccine designed for efficacy

against the latent stage of infection. A combination of a prophylactic vaccine and a therapeutic vaccine, which is active against both primary and latent infection, constitutes a multiphase vaccine.

Due to genetic variation, different individuals may react with immune responses of varying strength to the same polypeptide. Therefore, the vaccine according to the invention may comprise several different polypeptides in order to increase the immune response. The vaccine may comprise two or more polypeptides or immunogenic portions, where all of the polypeptides are as defined above, or some but not all of the peptides may be derived from virulent mycobacteria. In the latter example, the polypeptides not necessarily fulfilling the criteria set forth above for polypeptides may either act due to their own immunogenicity or merely act as adjuvants.

The vaccine may comprise 1-20, such as 2-20 or even 3-20 different polypeptides or fusion polypeptides, such as 3-10 different polypeptides or fusion polypeptides.

The invention also pertains to a method for immunizing an animal, including a human being, against TB caused by virulent mycobacteria, comprising administering to the animal the polypeptide of the invention, or a vaccine composition of the invention as described above, or a living vaccine described above.

The invention also pertains to a method for producing an immunologic composition according to the invention, the method comprising preparing, synthesizing or isolating a polypeptide according to the invention, and solubilizing or dispersing the polypeptide in a medium for a vaccine, and optionally adding other *M. tuberculosis* antigens and/or a carrier, vehicle and/or adjuvant substance.

DNA Vaccine.

The nucleic acid fragments of the invention may be used for effecting *in vivo* expression of antigens, *i.e.* the nucleic acid fragments may be used in so-called DNA vaccines as reviewed in Ulmer et al 1993, which is included by reference.

Hence, the invention also relates to a vaccine comprising a nucleic acid fragment according to the invention, the vaccine effecting *in vivo* expression of antigen by an animal, including a human being, to whom the vaccine has been administered, the

amount of expressed antigen being effective to confer substantially increased resistance to infections caused by virulent mycobacteria in an animal, including a human being.

The above mentioned definitions and distinctions of prophylactic-, booster-, therapeutic- and multiphase vaccines also applies for DNA vaccines

The efficacy of such a DNA vaccine can possibly be enhanced by administering the gene encoding the expression product together with a DNA fragment encoding a polypeptide that has the capability of modulating an immune response.

Live recombinant vaccines

One possibility for effectively activating a cellular immune response for a vaccine can be achieved by expressing the relevant antigen in a vaccine in a non-pathogenic microorganism or virus. Well-known examples of such microorganisms are *Mycobacterium bovis* BCG, *Salmonella* and *Pseudomona* and examples of viruses are Vaccinia Virus and Adenovirus.

Therefore, another important aspect of the present invention is an improvement of the living BCG vaccine presently available, wherein one or more copies of a DNA sequence encoding one or more polypeptide as defined above has been incorporated into the genome of the micro-organism in a manner allowing the micro-organism to express and secrete the polypeptide. The incorporation of more than one copy of a nucleotide sequence of the invention is contemplated to enhance the immune response.

Another possibility is to integrate the DNA encoding the polypeptide according to the invention in an attenuated virus such as the vaccinia virus or Adenovirus (Rolph et al 1997). The recombinant vaccinia virus is able to replicate within the cytoplasma of the infected host cell and the polypeptide of interest can therefore induce an immune response, which is envisioned to induce protection against TB.

Therapeutic vaccine.

The invention also relates to the use of a polypeptide or nucleic acid of the invention for use as therapeutic vaccines as have been described by D. Lowrie (Lowrie, 1999)

using DNA vaccine encoding HSP65 from *M. leprae*. Antigens with therapeutic properties may be identified based on their ability to diminish the severity of *M. tuberculosis* infection in experimental animals or prevent reactivation of previous infection, when administered as a vaccine. The composition used for therapeutic vaccines can be prepared as described above for vaccines.

Diagnostic protein

The invention also relates to a method of diagnosing latent TB caused by a virulent mycobacterium in an animal, including a human being, comprising intradermally injecting, in the animal, a polypeptide according to the invention, a positive skin response at the location of injection being indicative of the animal having TB, and a negative skin response at the location of injection being indicative of the animal not having TB.

When diagnosis of latent infection with virulent mycobacteria is the aim, a blood sample comprising mononuclear cells (*i.e.* T-lymphocytes) from a patient is contacted with a sample of one or more polypeptides of the invention. This contacting can be performed *in vitro* and a positive reaction could e.g. be proliferation of the T-cells or release of cytokines such as IFN-γ into the extracellular phase. It is also conceivable to contact a serum sample from a subject with a polypeptide of the invention, the demonstration of a binding between antibodies in the serum sample and the polypeptide being indicative of previous or ongoing infection.

The invention therefore also relates to an *in vitro* method for diagnosing latent infection in an animal or a human being with a virulent mycobacterium, the method comprising providing a blood sample from the animal or human being, and contacting the sample from the animal with the polypeptide of the invention, a significant release into the extracellular phase of at least one cytokine by mononuclear cells in the blood sample being indicative of the animal being sensitized. A positive response being a response more than release from a blood sample derived from a patient without the TB diagnosis plus two standard deviations. The invention also relates to the *in vitro* method for diagnosing ongoing or previous sensitization in an animal or a human being with a virulent mycobacterium, the method comprising providing a blood sample from the animal or human being, and by contacting the sample from the animal with

the polypeptide of the invention demonstrating the presence of antibodies recognizing the polypeptide of the invention in the serum sample.

The immunogenic composition used for diagnosing may comprise 1-20, such as 2-20 or even 3-20 different polypeptides or fusion polypeptides, such as 3-10 different polypeptides or fusion polypeptides.

Diagnostic DNA

The nucleic acid probes encoding the polypeptide of the invention can be used in a variety of diagnostic assays for detecting the presence of pathogenic organisms in a given sample.

A method of determining the presence of mycobacterial nucleic acids in an animal, including a human being, or in a sample, comprising administering a nucleic acid fragment of the invention to the animal or incubating the sample with the nucleic acid fragment of the invention or a nucleic acid fragment complementary thereto, and detecting the presence of hybridized nucleic acids resulting from the incubation (by using the hybridization assays which are well-known in the art), is also included in the invention. Such a method of diagnosing TB might involve the use of a composition comprising at least a part of a nucleotide sequence as defined above and detecting the presence of nucleotide sequences in a sample from the animal or human being to be tested which hybridize with the nucleic acid fragment (or a complementary fragment) by the use of PCR technique.

Antibodies

A monoclonal or polyclonal antibody, which is specifically reacting with a polypeptide of the invention in an immunoassay, or a specific binding fragment of said antibody, is also a part of the invention. The antibodies can be produced by methods known to a person skilled in the art. Polyclonal antibodies can be raised in a mammal, for example, by one or more injections of a polypeptide according to the present invention and, if desired, an adjuvant. The monoclonal antibodies according to the present invention may, for example, be produced by the hybridoma method first described by Kohler and Milstein (Kohler and Milstein, 1975), or may be produced by recombinant DNA methods such as described in U.S. Pat. No. 4,816,567. The monoclonal anti-

bodies may also be isolated from phage libraries generated using the techniques described by McCafferty et al (McCafferty, 1990), for example. Methods for producing antibodies are described in the literature, e.g. in US6136958.

A sample of a potentially infected organ or body fluid from an infected individual may be contacted with such an antibody recognizing a polypeptide of the invention. The demonstration of the reaction by means of methods well known in the art between the sample and the antibody will be indicative of an ongoing infection. It is of course also a possibility to demonstrate the presence of anti-mycobacterial antibodies in serum or other body fluids by contacting a serum sample from a subject with at least one of the polypeptide fragments of the invention and using well-known methods for visualizing the reaction between the antibody and antigen.

In diagnostics, an antibody, a nucleic acid fragment and/or a polypeptide of the invention can be used either alone, or as a constituent in a composition. Such compositions are known in the art, and comprise compositions in which the antibody, the nucleic acid fragment or the polypeptide of the invention is coupled, preferably covalently, to at least one other molecule, e.g. a label (e.g. radioactive or fluorescent) or a carrier molecule.

It will be understood that the following examples are illustrative of the present invention and are not a limitation thereof. A number of variations on the techniques, reagents, and conditions described in the following examples will be readily apparent to one of skill in the art.

Examples

EXAMPLE 1: Cloning and expression of low oxygen induced M. tuberculosis antigens in *E. coli*.

A number of M tuberculosis genes are induced under low oxygen conditions. The up-regulation of the genes listed in table 2 has been determined at either the mRNA (Sherman, 2001) or protein (Boon, 2001, Rosenkrands, 2002) level. The coding region of these selected antigens is amplified by PCR using the primer sets listed in table 3.

Table 3. Prim r sequences for PCR amplification of selected low oxygen induced antigens

Rv no.		SEQ ID NO:	Primer sequence
Rv0079	Fwd	92	CACCGTGGAACCGAAACGCAGTCG
	Rvs	93	TTATGCCAGACCGTCGGCA
Rv0080	Fwd	94	CACCATGAGCCCGGGCTCG
	Rvs	95	TTACGGCGTACGCGAGTCAG
Rv0081	Fwd	96	CACCGTGGAGTCCGAACCGCTGTA
	Rvs	97	TTACGTGGCCGAGCCGC
Rv0363c (*fba*)	Fwd	98	CACCATGCCTATCGCAACGCCC
	Rvs	99	TTAGTGGGTTAGGGACTTTCCGG
Rv0569	Fwd	100	GGGGACAAGTTTGTACAAAAAAGCAGGCTTAAAGGCAAAGGTCGGGGAC
	Rvs	101	GGGGACCACTTTGTACAAGAAAGCTGGGTCCTACGTTCCCCTGGCATGGA
Rv0572c	Fwd	102	CACCATGGGTGAGCACGCCATC
	Rvs	103	TTATAGGTCATCGGATTGAGGTGATC
Rv0574c	Fwd	104	CACCGTGGCTGGCAATCCTGATGT
	Rvs	105	TTACTCCTTGCTCGTTAGGTTGGC
Rv1264	Fwd	106	CACCGTGACAGACCACGTGCGC
	Rvs	107	TTACGGTGACGAGCCGGC
Rv1592c	Fwd	108	CACCATGGTAGAGCCCGGCAATTTG
	Rvs	109	TTAGAGCGGACGGCGGCT
Rv1733c	Fwd	110	CACCATGATCGCCACAACCCGC
	Rvs	111	TTACCGCTGCGTGCAGAACA
Rv1734c	Fwd	112	CACCATGACCAACGTCGGTGACCA
	Rvs	113	TTATCCTGTTACTGCGGCGCA
Rv1736c (*narX*)	Fwd	114	CACCGTGACGGTGACACCACGGAC
	Rvs	115	TTACCACCCGCGCCGC
Rv1737c (*narK2*)	Fwd	116	CACCATGAGAGGGCAAGCGGC
	Rvs	117	TTACCTGGACGCCTCCTCACTC
Rv1738	Fwd	118	CACCATGTGCGGCGACCAGTC
	Rvs	119	TTAATACAACAATCGCGCCGG
Rv1739c	Fwd	120	CACCATGATTCCCACGATGACATCG
	Rvs	121	TTAGCGCCGACGGAACG
Rv1813c	Fwd	122	GGGGACAAGTTTGTACAAAAAAGCAGGCTTAATCACAAACCTCCGACGC
	Rvs	123	GGGGACCACTTTGTACAAGAAAGCTGGGTCCTAGTTGCACGCCCAGTTGAC
Rv1997 (*ctpF*)	Fwd	124	CACCTTGTCGGCGTCAGTGTCTGC
	Rvs	125	TTATGGCGGTTGCGCCC
Rv1998c	Fwd	126	CACCATGAGTTTCCACGATCTTCATCACC
	Rvs	127	TTACGTTGTACTCGTGCGGTTCTC
Rv2003c	Fwd	128	CACCGTGGTCAAGCGCTCTCGG
	Rvs	129	TTATTCCGACTCGAGTGGGTGA

Table 3 (cont'd)

Rv no.		SEQ ID NO:	Primer sequence
Rv2005c	Fwd	130	CACCATGTCTAAACCCCGCAAGCA
	Rvs	131	TTACGACTGCCGTGCCACG
Rv2007c	Fwd	132	CACCGTGACCTATGTGATCGGTAGTGAGTG
(*fdxA*)	Rvs	133	TTAAGGGCACTCCACCGGGA
Rv2028c	Fwd	134	CACCATGAACCAATCACACAAACCCC
	Rvs	135	TTACAGATACTGCTGACCGACGACC
Rv2029c	Fwd	136	CACCATGACGGAGCCAGCGG
(*pfkB*)	Rvs	137	TTATGGCGAGGCTTCCGG
Rv2030c	Fwd	138	GGGGACAAGTTTGTACAAAAAAGCAGGCTTACTGATGACCGCAGCGGCT
	Rvs	139	GGGGACCACTTTGTACAAGAAAGCTGGGTCCTACAGACCGGTCGGG-TAGGTTT
Rv2031c	Fwd	140	GGGGACAAGTTTGTACAAAAAAGCAGGCTTAGCCACCACCCTTCCCGT
(*hspX*)	Rvs	141	GGGGACCACTTTGTACAAGAAAGCTGGGTCCTAGTTGGTGGACCGGATCT-GAAT
Rv2032	Fwd	142	CACCATGCCGGACACCATGGTG
	Rvs	143	TTAGTGATCCTTAGCCCGAACGTG
Rv2428	Fwd	144	GGGGACAAGTTTGTACAAAAAAGCAGGCTTAATGCCACTGCTAACCATTGGC
(*ahpC*)	Rvs	145	GGGGACCACTTTGTACAAGAAAGCTGGGTCCTAGGCCGAAGCCTTGAGGAGT
Rv2624c	Fwd	146	CACCATGTCTGGGAGAGGAGAGCCG
	Rvs	147	TTAGCGAACGACAAGCACCGA
Rv2625c	Fwd	148	GGGGACAAGTTTGTACAAAAAAGCAGGCTTACGTGATGCGATCCCGCT
	Rvs	149	GGGGACCACTTTGTACAAGAAAGCTGGGTCCTACCCCGCATCGGAAAACC
Rv2627c	Fwd	150	GGGGACAAGTTTGTACAAAAAAGCAGGCTTAATGGCAAGTTCTGCGAGCGA
	Rvs	151	GGGGACCACTTTGTACAAGAAAGCTGGGTCCTAGGAACGGTCGCGCTGTGT
Rv2628	Fwd	152	CACCATGTCCACGCAACGACCG
	Rvs	153	TTAACCGCAACGGCAATCTCA

Table 3 continued			
Rv no.		**SEQ ID NO:**	**Primer sequence**
Rv2629	Fwd	154,	CACCATGCGATCAGAACGTCTCCG
	Rvs	155,	TTAGGATCTATGGCTGCCGAGTC
Rv2630	Fwd	156,	CACCATGCTGCACCGCGACGA
	Rvs	157,	TTACACATCGAGCGTTACCGCAC
Rv2659c	Fwd	158,	GGGGACAAGTTTGTACAAAAAAGCAGGCTTAGTGACGCAAACCGGCAA
	Rvs	159,	GGGGACCACTTTGTACAAGAAAGCTGGGTCCTACATCTCCTGGTTCTCGGCC
Rv2780	Fwd	160,	GGGGACAAGTTTGTACAAAAAAGCAGGCTTACGCGTCGGTATTCCGACC
	Rvs	161,	GGGGACCACTTTGTACAAGAAAGCTGGGTCCTACACGCTGGCGGGCTC
Rv3126c	Fwd	162,	CACCATGGTCATCCGGTTTGATCAAATA
	Rvs	163,	TTATGGATTGTCAATGACAGCCCA
Rv3127	Fwd	164,	CACCGTGCTCAAGAACGCAGTCTTGC
	Rvs	165,	TTAAGGCGGGCTGAACCAACC
Rv3128c	Fwd	166,	CACCGTGTGGTCCGCCTCGG
	Rvs	167,	TTAGCCCGCCTTGATCAGGA
Rv3129	Fwd	168,	CACCGTGGTGCAAGGCCGCA
	Rvs	169,	TTATCGCTGGTTGTGTGACGAG
Rv3130c	Fwd	170,	CACCATGAATCACCTAACGACACTTGACG
	Rvs	171,	TTACACAACCAGCGATAGCGCTC
Rv3131	Fwd	172,	CACCATGAACACCCATTTCCCGG
	Rvs	173,	TTAGCACCGTTGTCGCAGTAGCT
Rv3132c	Fwd	174,	CACCATGACAACAGGGGGCCTCG
	Rvs	175,	TTACTGCGACAACGGTGCTGAC
Rv3134c	Fwd	176,	CACCATGAGCGATCCTCGGCCA
	Rvs	177,	TTACAAGTTGGCACTGCGTACCG
Rv3841 (*bfrB*)	Fwd	178,	CCGGCTGAGATCTATGACAGAATACGAAGGGC
	Rvs	179,	CCCCGCCAGGGAACTAGAGGCGGC
Rv3842c (*glpQ1*)	Fwd	180,	CACCATGACATGGGCCGACGAG
	Rvs	181,	TTAGCGAGTGGTCCCGTTCG
Rv3908	Fwd	182,	CACCGTGTCCGACGGCGAACAA
	Rvs	183,	TTACGGCCCCGGCCC

PCR reactions were carried out using Platinum Tag DNA Polymerase (GIBCO BRL) in a 50 μl reaction volume containing 60 mM Tris-SO_4 (pH 8.9), 18 mM Ammonium Sulfate, 0.2 mM of each of the four nucleotides, 0.2 μM of each primer and 10 ng of M. tuberculosis H37Rv chromosomal DNA. The reaction mixtures were initially heated to 95° C for 5 min., followed by 35 cycles of: 95° C for 45 sec, 60° C for 45 sec and 72° C for 2 min. The amplification products were precipitated by PEG/$MgCl_2$, and dissolved in 50 μL TE buffer.

DNA fragments were cloned and expressed in Gateway Cloning system (Life Technology). First, to create Entry Clones, 5 μL of DNA fragment was mixed with 1 μL of pDONR201, 2 μL of BP CLONASE enzyme mix and 2 μL of BP reaction buffer. The recombination reactions were carried out at 25° C for 60 min. After Proteinase K treatment at 37° C for 10 min., 5 μL of each sample was used to transform *E. coli* DH5α competent cells. Transformants were selected on LB plates containing 50 μg/mL kanamycin. One bacterial clone from each transformation was grown in 3 mL LB medium containing 50 μg/mL kanamycin and plasmid DNA was isolated (Qiagen).

Second, to create expression clones, 2 μL of each entry clone DNA was mixed with 1 μL of His-tagged expression vector (pDest17), 2 μL LR reaction buffer, 2μL LR CLONASE enzyme mix and 3 μL TE. After recombination at 25° C for 60 min. and Proteinase K treatment at 37° C for 10 min., 5 μL of each sample was used to transform *E. coli* BL21-SI competent cells. Transformants were selected on LBON (LB without NaCl) plates containing 100 μg/mL ampicillin. The resulting E. coli clones express recombinant proteins carrying a 6-histine tag at the N-terminal. All clones were confirmed by DNA sequencing.

Recombinant proteins were purified from transformed E. coli BL21-SI cells cultured in 900 mL LBON medium containing 100 μg/mL at 30° C until OD_{600} = 0.4-0.6. At this point 100 mL 3 M NaCl was added and 3 hours later bacteria were harvested by centrifugation. Bacteria pellets were resuspended in 20 mL bacterial protein extraction reagent (Pierce) incubated for 10 min. at room temperature and pelleted by centrifugation. Bacteria were lysed and their DNA digested by treating with lysozyme (0.1 mg/mL) and DNase I (2.5 μg/mL) at room temperature for 30 minutes, with gentle agitation. The recombinant protein forms inclusion bodies and can be pelleted by centrifugation at 27.000 x g for 15 min. Protein pellets were solubilized by adding 20 ml of sonication buffer (8 M urea, 50 mM Na_2HPO_4, 100 mM Tris-HCl, pH 8.0) and sonicating 5 x 30 sec pulses interrupted by a 30 sec pause. After another centrifugation at 27.000 x g for 15 min., supernatants were applied to 10 mL TALON columns (Clontech). The columns were washed with 50 mL sonication buffer. Bound proteins were eluted by lowering pH (8 M urea, 50 mM Na_2HPO_4, 100 mM Tris-HCl, pH 4.5). 5 mL fractions were collected and analyzed by SDS-PAGE. Fractions containing re-

combinant protein were pooled. Further purification was achieved by anion- or cation-exchange chromatography on Hitrap columns (Pharmacia). Bound protein was eluted using a NaCl gradient from 0 – 500 mM in 3 M urea, 10 mM Tris-HCl, pH 8.0. All fractions were collected and analyzed on SDS-PAGE using Coomassie staining. Fractions containing recombinant protein were pooled. Final protein concentrations were determined by micro BCA (Pierce).

EXAMPLE 2: Prophylactic versus therapeutic vaccine.
Murine vaccination models.

A prophylactic vaccine given prior to infection should induce an immune response sufficiently strong to prevent or dampen the initial proliferation of the bacteria in the acute phase and thereby reduce the ensuing disease. In the murine prophylactic vaccine model outlined in figure 1A, naïve mice are immunized 3 times, 2 weeks apart with recombinant antigens. Six weeks after the last immunization, the mice are given an aerosol infection with approximately 250 *M. tuberculosis* bacilli. The protective capacity of the vaccine is evaluated by enumeration of the bacteria in spleen and lung 6 weeks post-infection.

To define the optimal components for a therapeutic vaccine, a murine reactivation model of latent TB has been established (van Pinxteren, 2000) (figure 1B). An aerosol infection with approximately 250 *M. tuberculosis* bacilli is given and at the peak of infection 6 weeks later, the mice receive an 8-week course of anti-mycobacterial drug treatment of isoniazid and rifabutin given in the drinking water. This reduces the bacterial load in spleen and lung to a low level (about 500 bacteria per organ). This latent phase of low chronic infection is stable for 9-10 weeks after which a slow spontaneous reactivation occurs. The therapeutic vaccine is given as 3 subcutaneous (s.c.) immunizations about 5 weeks after cessation of drug treatment. The effect of the therapeutic vaccine is evaluated as protection against reactivation determined by enumeration of bacteria in spleen and lung 7 weeks after the last immunization.

The effect of the antigens in a prophylactic or a therapeutic vaccine.
BCG, ESAT6, and Rv2031c, one of the most prominent proteins induced under low oxygen conditions (Rosenkrands, 2002), were analyzed for their prophylactic and therapeutic vaccine potential. Naïve or latently infected C57Bl mice were immunized

with one s.c. injection of $2.5x10^5$ BCG, or 3 s.c. immunizations of 10μg of either recombinant ESAT6 or recombinant Rv2031c in a DDA/MPL adjuvant. The vaccinations were done in groups of 5 mice and protective capacity of the vaccines was evaluated as described above. Figure 2 shows the bacterial load in the lung in the acute phase (A) and in the reactivation phase (B), after prophylactic and therapeutic vaccination respectively. ESAT6 (as previously described by Brandt, 2000) offers protection against acute phase infection at the level of BCG (figure 2A). However, neither of the two shows any protective effect against reactivation of the infection when given during latent infection (figure 2B). In contrast, Rv2031c, the low oxygen induced antigen, offers no protection against the acute phase of the infection when given as a prophylactic vaccine, but gives some protection against reactivation when given as a therapeutic vaccine. That is, some antigens, here exemplified by ESAT6, though potent as prophylactic vaccines have no effect as therapeutic vaccines. In contrast, other antigens, here exemplified by Rv2031c, can be efficient therapeutic vaccines although they have no effect or only negligible effect as prophylactic vaccines.

Example 3. Low oxygen induced antigens, Rv2031c, as therapeutic vaccines: There is a high variability in bacterial load intrinsic to the reactivation model in the latent and reactivation phase. The analysis of Rv2031c as a therapeutic vaccine was therefore repeated in groups of eight mice. As in the previous experiments the mice were given 3 s.c. immunizations of 10μg rRv2031c in DDA/MPL. The induced immune responses were analyzed one week post immunization. The mice were partially bled and the PBMC from the blood purified and analyzed for Rv2031c- and ESAT6 specific recall responses. Using ELIspot technique, the frequency of Rv2031c-specific and ESAT6-specific IFN-γ-producing cells were determined in both the rRv2031c immunized and the unimmunized group (figure 3). The rRv2031c immunization has increased the frequency of Rv2031c-specific IFN-γ producing cells by a factor of 43 as compared to the unimmunized group. In contrast, the frequency of ESAT6-specific IFN-γ producing cells is significantly higher in the unimmunized group. ESAT6 is an antigen produced in high amounts by the actively-growing *M. tuberculosis* bacteria. The level of the ESAT6 specific immune response in infected mice could therefore be indicative the degree of actively-growing infection in the animals. Recent reports have in fact demonstrated such a correlation between the level

of ESAT6 response and degree of disease in both *M. tuberculosis*-infected humans and *M. bovis*-infected cattle (Doherty, 2002, Vordermeier, 2002). Therefore, the higher ESAT6 response in the unimmunized group of latently-infected mice could be indicative of a transition into the reactivation phase, where the bacteria are again beginning to multiply.

To analyze the epitope recognition pattern of Rv2031c, fourteen overlapping peptides (each 20 amino acids long) covering the whole Rv2031c protein were synthesized. Initially the peptides were analyzed in 4 pools of 3-4 peptides. PBMCs from rRv2031c immunized latently-infected mice were incubated with the peptide pools (5 μg/ml per peptide) for 72 h. The specific peptide-induced IFN-γ production was quantitated in the supernatant in a standard sandwich ELISA using paired anti-murine IFN-γ antibodies (PharMingen) and recombinant IFN-γ (PharMingen) as standard. Both peptide pool 1-4 and 8-10 stimulated a significant IFN-γ response (figure 4A). The individual peptides of these two pools were re-analyzed (figure 4B). This clearly shows that the response to Rv2031c contains a dominant epitope, peptide 2 (PRSLFPEFSELFAAFPSFAG, aa 11-30 of SEQ ID NO:24), and a subdominant epitope, peptide 9 (RTEQKDFDGRSEFAYGSFVR, aa 81-100 of SEQ ID NO:24).

The therapeutic effect of the rRv2031c immunizations was analyzed 7 weeks after the last immunization. Figure 5 shows the bacterial load in the lung (A) and the spleen (B) of both rRv2031c-immunized and unimmunized mice. There is a clear reduction in the level of bacteria in both organs in the rRv2031c-immunized group. That is, the induction of Rv2031c T cell responses can participate in keeping the latent infection in check.

Example 4. Low oxygen induced antigens, Rv0569, as therapeutic vaccines

Rv0569 is also a low oxygen induced antigen described in WO0179274 and illustrates very well the potential as a therapeutic vaccine.

We have established a murine reactivation model of latent TB [van Pinxteren et al, 2000, 30:3689-98], which is a variant of the so-called Cornell model. An aerosol infection is allowed to be established and at the peak of infection 6 weeks after, the mice receive an 8-week course of anti-mycobacterial drug treatment of isoniazid and rifabutin

given in the drinking water. This reduces the bacterial load in spleen and lung to a low level. This latent phase of low chronic infection is stable for 9-10 weeks after which a slow spontaneous reactivation can be detected. This model is used to analyze the protective effect of a post exposure vaccine on reactivation.

Rv0569, which is highly up regulated under low oxygen growth conditions [Rosenkrands et al, 2002, 184(13): 3485-91], was analyzed for its ability to protect against reactivation given as a therapeutic vaccine in the latent phase of TB infection. Latent infected C57Bl mice were vaccinated with 3 s.c. injections of 3 μg recombinant Rv0569 and for comparison with 3 s.c. injections of 3 μg recombinant ESAT6 or one s.c. injection of BCG. The effect of the vaccine is evaluated 7 weeks after the last immunization. The induced immune responses were analyzed for Rv0569 or ESAT6 specific responses in an in vitro recall assay. Isolated splenocytes were incubated with 1μg/ml of Rv0569 or 1μg/ml of ESAT6 for 72h. The produced IFNγ in the culture supernatant was quantitated in a standard sandwich ELISA. Figure 6 shows a nice Rv0569 specific IFNγ response induced in the Rv0569 vaccinated group with practically no response in the un-vaccinated group. The ESAT6 vaccination enhanced the ESAT6 specific response though a significant ESAT6 response persisted in the un-vaccinated latent infected group.

The Rv0569 induced protection against reactivation was determined by enumeration of bacteria in spleen and lung 7 weeks after the last immunization. Figure 7 shows the bacterial load in the lung and the spleen of both Rv0569-vaccinated, ESAT6-vaccinated, BCG vaccinated and un-vaccinated latently infected mice. There is a clear reduction in the level of viable bacteria in both spleen and lungs of the Rv0569 vaccinated mice, whereas neither ESAT6 nor BCG are able to inhibit the growth of the M. tuberculosis bacteria when given as a vaccine during latent infection. That is, the induction of Rv0569 T cell responses can participate in keeping the latent infection in check.

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All documents cited above are incorporated by reference herein. A variety of modifications and variations on the processes, conditions, reagents and compositions described herein will be readily apparent to one of skill in the art given the teachings of the present invention. Such modifications and variations are within the scope of the invention as set forth in the following claims.